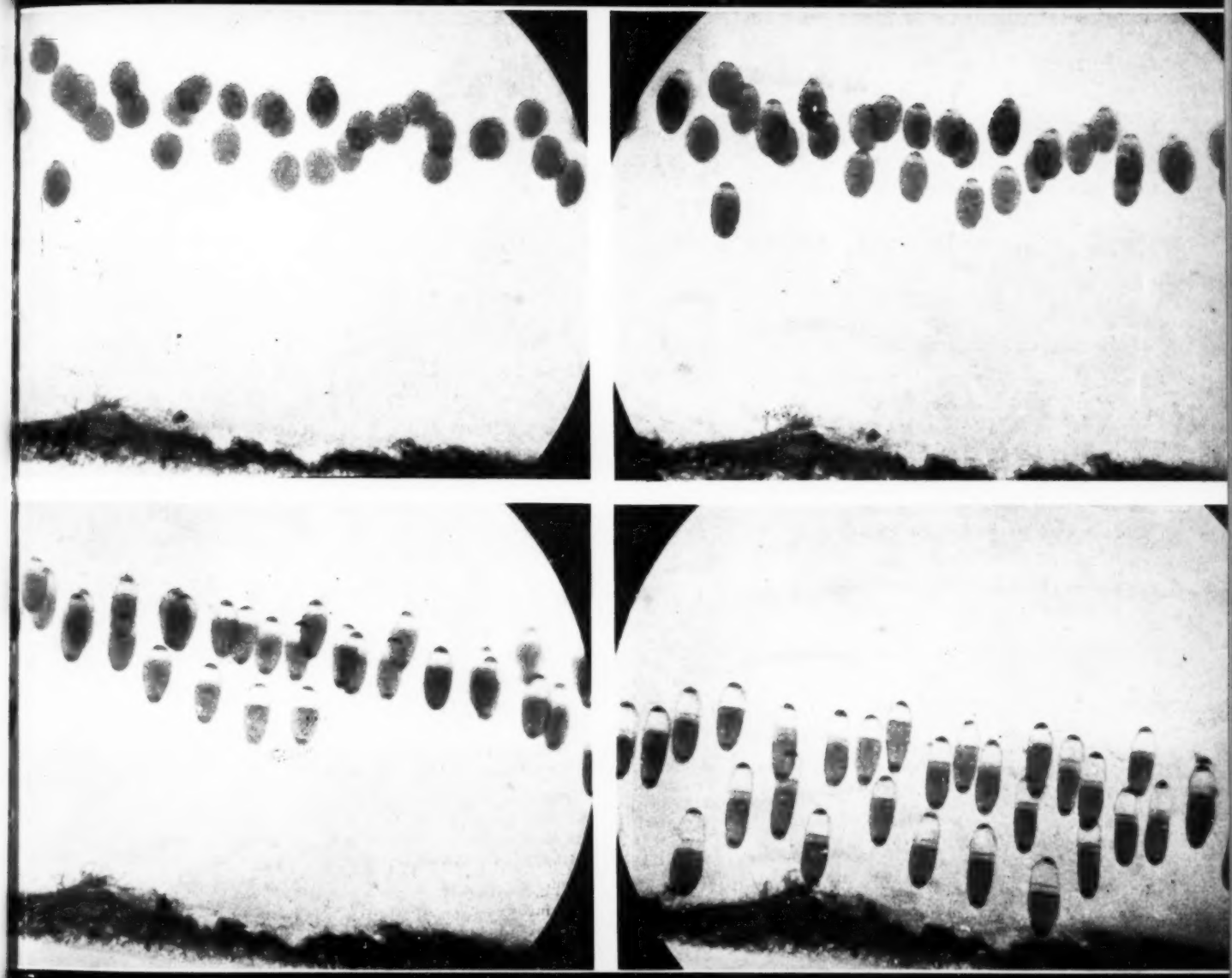
JOURNAL OF APPLIED PHYSICS



JOURNAL OF APPLIED

PHYSICS

(Formerly PHYSICS)

FEBRUARY, 1938 Vol. 9, No. 2

In This Issue

Page

Applications of Physics in Biology H. A. B. 67

Some Physical Properties of Protoplasm E. Newton Harvey 68

Diffusion Processes in Living Systems M. H. Jacobs 81

Electrical Signs of Biological Activity Herbert S. Gasser 88

Potential Energies of Biologically Important Oxidation-Reduction Systems William Mansfield Clark 97

Optical Studies of the Molecular Organization of Living Systems Francis O. Schmitt 109

Contributed Original Research

Experimental Analysis of Rein's Thermostromuhr for Small Flows
J. F. Herrick, Edward J. Baldes and F. P. Sedgwick 124

Condensation and Adsorption of Mercury Vapor
R. C. Mason 131

Photographic Study of Lightning
R. E. Holzer, E. J. Workman and L. B. Snoddy 134

In Every Issue

Here and There 118

Innovations in Instruments 120

Some of the New Books 122

Among Our Contributors 123

Current Literature of Physics viii

Advertising Index xx

BOARD OF EDITORS

Elmer Hutchisson,
Editor

Associate Editors

R. M. Bozorth
D. W. Bronk
W. P. Davey
O. S. Duffendack
Saul Dushman
A. Stuart Hunter
C. H. Kunsman
J. T. Littleton
L. L. Nettleton
W. B. Nottingham
A. R. Olpin
John T. Tate

Publication of the American Institute of Physics

ADMINISTRATIVE STAFF

Henry A. Barton,
Director

Madeline M. Mitchell,
Publications Manager

William C. Copp,
Advertising Manager

GOVERNING BOARD

John T. Tate, *Chairman*
Karl T. Compton
E. C. Crittenden
Wheeler P. Davey
Homer L. Dodge
Harvey Fletcher
Paul E. Klopsteg
Vern O. Knudsen
Melvin Mooney
Frederic Palmer, Jr.
R. L. Peek, Jr.
George B. Pegram
W. B. Rayton
F. K. Richtmyer
Wallace Waterfall

The Journal of Applied Physics, published monthly at Prince and Lemon Sts., Lancaster, Pa., is devoted to physics in its role as the science basic to other natural sciences and to the arts and industries. Previous to 1937 this publication was known as Physics. It aims to be of service not only in physical laboratories, but also in the laboratories of industrial, chemical, geological, geophysical, meteorological, radio, and similar concerns. The Journal of Applied Physics publishes editorials, news of physicists and reviews, as well as technical papers of applied physics.

SUBSCRIPTION PRICE

	U. S., POSS. AND CANADA	FOREIGN
To members of scientific societies	$5.00	$5.70
To all others	7.00	7.70

Manuscripts should be submitted to Elmer Hutchisson, Editor, University of Pittsburgh, Pittsburgh, Pennsylvania.

Changes of address, proofs, and complaints of failure to receive the Journal of Applied Physics should be addressed to the Publications Manager. New copies can be sent free only if notice of nondelivery is received within three months of date of issue.

Subscriptions and orders for back numbers should be addressed to Prince and Lemon Streets, Lancaster, Pa. or to the American Institute of Physics, 175 Fifth Avenue, New York, N. Y.

Advertising rates supplied on request. Orders, advertising copy and cuts should be sent to the American Institute of Physics.

Entered as second class matter at the Post Office at Lancaster, Pennsylvania, under the Act of March 3, 1897.
Accepted for mailing at the special rate of postage provided for in the Act of February 28, 1925, authorized May 2, 1932.

Copyright 1938, by the American Institute of Physics.

Journal of Applied Physics

Volume 9 February, 1938 Number 2

APPLICATIONS OF PHYSICS IN BIOLOGY

A SYMPOSIUM on biophysics, the first large meeting devoted to this borderland field, was held by the American Institute of Physics in cooperation with the Eldridge Reeves Johnson Foundation for Medical Physics of the University of Pennsylvania on November 4, 5, and 6 in Philadelphia. The meeting place was the University Museum.

The meeting served two principal purposes. It marked a stage in the progress of those fields of biological research in which the methods and results of physical research play a large roll, and it provided a forum where research men of this field might meet. The program reflected this twofold character. The first two days were devoted to invited papers by twelve leaders of research. Their topics ranged from those almost exclusively of interest to biologists to others which are of as great interest to the pure physicist as to the biologist. In the evenings of these two days, Dr. Irving Langmuir presented the first two of his Johnson Foundation lectures on "Monolayers and Multilayers and Their Applications to Biological Problems." The third day of the meeting consisted of two parallel sessions in which some thirty short papers were presented on the subject of current researches and their results.

Throughout the three days of sessions there was a large attendance. It is a tribute to the speakers and the manner in which they presented their interesting and important subjects that the audience was unusually attentive and almost without exception remained in the auditorium for each entire session without intermission. The number registered as attending the symposium was 350, but guests, students and other auditors swelled this total at some sessions to about 800.

The biophysics symposium is the second of a series to be sponsored by the American Institute of Physics on various fields of applied physics. The first dealing with physics in metals was held in January, 1937, at Cambridge, Massachusetts. A third, concerned with the applications of physics in automotive transportation is planned for the near future.

The present issue of this journal contains several papers from the biophysics symposium. Other papers from this important field of applied physics will appear in next month's issue.

—H. A. B.

For March

Electron Diffraction Methods of Studying Organic Films, by L. H. GERMER

Biophysics and Biochemistry of Viruses, by W. M. STANLEY

Photochemical Basis of Vision, by S. HECHT

Mechanics of Muscular Contraction in Man, by W. O. FENN

Some Aspects of Nuclear Physics of Possible Interest in Biological Work, by L. A. DUBRIDGE

Some Physical Properties of Protoplasm*

By E. NEWTON HARVEY

Princeton University
Princeton, New Jersey

Introduction

THE word protoplasm, coming from the Greek, *protos*, first, and *plasma*, a thing formed, means literally "the first creation." It was used by Purkinje in 1840 for the formative material of the animal embryo and by von Mohl in 1846 for the contents of a plant cell. Huxley (1868) called it the "physical basis of life" and argued that "if properties of water result from the nature and disposition of its molecules, I can find no ground for refusing to say that the properties of protoplasm result from the nature and disposition of its molecules."

Protoplasm was generally considered "an albuminous substance containing carbon, hydrogen, oxygen and nitrogen in extremely complex and unstable molecular combination and capable under proper conditions of manifesting certain vital phenomena, as spontaneous motion, sensation, assimilation and reproduction;" or, "protoplasm from whatever source . . . is essentially uniform and similar in appearance and properties . . . that of a grayish, viscid, slimy, semitransparent and semifluid substance." Amoeba was regarded as the simplest example of "primitive living protoplasm," a "sort of slime." The idea that protoplasm was a substance, "the living substance," persisted and in the latter part of the 19th century there was a great controversy as to whether its structure was fibrillar, alveolar or granular. These views were all correct in that the contents of different living cells may have any of the above types of structure but incorrect in the idea that protoplasm is a substance.

The modern view is to regard the cell as a *system*, a mixture of many different substances, often separated from each other by phase boundaries or membranes, whose mutual interaction results in the varied phenomena we associate with life. No one of these substances can be considered as "living" except insofar as it is indispensable for the continuance of life. On this view salts are as fundamental a part of the make up of protoplasm as are proteins, for no cell can function in complete absence of salts. Properties of protoplasm is an incorrect although a convenient phrase.

Biophysical study of the cell (using the word cell rather than protoplasm) means applying the knowledge of physics to explain what we observe living things to do. Movement, transmission of nerve impulses, reception of stimuli by sense organs, energy transformations, absorption of material, reproduction, are all represented on this program. The great majority of investigations involve applying the principles of classical physics but because of the small size of cells the approach must be by the micro method and the problem is always more complicated than the physicist is accustomed to deal with. Consequently the accuracy of quantitative work is not so great and often we must be satisfied with order of magnitude.

* Presented at the Symposium on Biophysics, Philadelphia, Pennsylvania, November 4, 5 and 6, 1937.

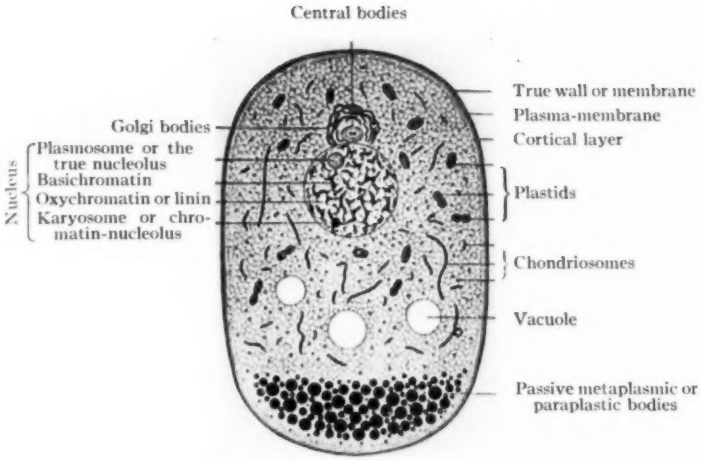


FIG. 1. Diagram of a resting cell showing the various structures which may be present.*

Where quantitative methods of a high degree of accuracy can be applied it is found that the same cell will vary from time to time depending on its previous history or almost indetectable changes in its environment, while different cells of the same tissue, although identical in appearance, possess properties which vary over a distribution curve whose limits may differ by a factor of two. Just as the molecules of a gas possess a mean kinetic energy at a given temperature, so a population of red blood cells may be said to have a mean permeability to water under definitely specified present conditions and also previous history.

A cell was defined by Leydig (1857) as a mass of protoplasm containing a nucleus, to which was later added the dictum that both cell and nucleus came from pre-existing cells and nuclei. To this we may further add that all cells possess a surface layer whose properties are specific and extraordinary. It was early observed that the aqueous contents of a cell did not mix with water until a surface layer, the "plasma membrane," had been destroyed.

This surface layer is spontaneously reformed when the cell contents are squeezed out through the original surface, a process which is dependent on the salt content of the medium, particularly the calcium content (Heilbrunn, 1928).[5] The lines of evidence in favor of a film at the cell surface are now so many and so striking that there can be no reasonable doubt of the existence of conditions there very different from the cell interior. The behavior in many respects resembles the behavior of a monomolecular layer, as has been emphasized by Langmuir[38] and others.

We may therefore consider the physical properties of a cell from the point of view (1) of its surface and (2) of its interior, thus:

Surface
- Tension
- Permeability and osmotic phenomena
- Electrical properties
  - Potential
  - Impedance

Interior
- Viscosity (gelation, thixotropy)
- Optical properties (birefringence, x-ray data)
- Energy changes

Since permeability of cells will be considered by Jacobs, cell potential by Gasser, cell impedance by Cole, optical properties by Schmitt and energy changes by Clark, I will limit my discussion of the properties of protoplasm to viscosity and tension at the surface.

A composite cell is represented in Fig. 1 as a diagram. Suspended in a fluid of the same osmotic pressure but not necessarily the same composi-

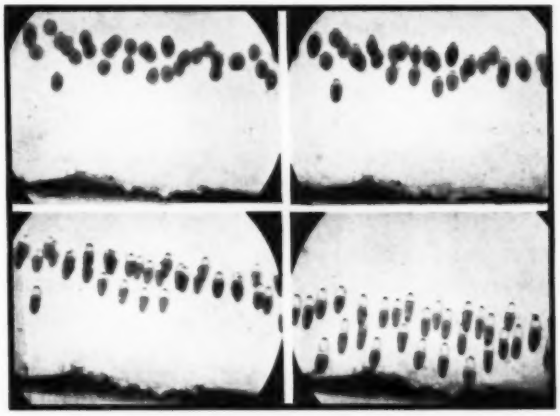


FIG. 2. Four stages in the separation of granules of different density in a sea urchin (Arbacia) egg subjected to a centrifugal force of 5500×gravity. Part of a moving picture taken through the centrifuge microscope; 7500 r.p.m. Exposure 0.8 second.

* After E. B. Wilson, *The Cell* (Macmillan, 1925).

tion or salt content, we have a system which can move, conduct impulses, metabolize, reproduce—exhibit all the manifestations of life. This system maintains a constant composition despite the continual flow of material and dissolved gases (O_2 and CO_2) in and out. It is the province of the biophysicist by studying properties, rates, equilibria, to explain how such a complex arrangement of membranes, phase boundaries, chromosomes, nucleoli, centrioles, asters, spindles, plastids, chondriosomes, Golgi apparatus, fibrillae, vacuoles, granules, alveoli and other structures can live and function.

In order to simplify the situation as much as possible it is convenient to select for study a cell of simple geometrical form, the sphere. Volume changes in hypo- and hypertonic solutions are easily calculated by measuring its diameter and thus its behavior as an osmometer can be investigated. Diffusion of substances into and out of spheres can be handled mathematically by much simpler equations than for cells of other shapes. The impedance of such spheres in suspension in salt solutions of known impedance can be determined by relatively simple relations based on the Maxwell equation (1873),[39] as can also the forces necessary to deform against the tension at their surface, which gives a means of calculating this tension.

The unfertilized eggs of marine animals are excellent spherical cells, around 70μ in diameter, whose properties have been extensively studied. These physical constants have been collected for the egg of a sea urchin, Arbacia punctulata (Harvey, 1932),[26] which is surrounded only by a jelly and a very thin pellicle both of which can be removed. Thus, no extraneous protecting membranes impede its behavior. Egg cells also possess the advantage that death or injury can be estimated by failure to develop or by partial development only, when they are fertilized. Special attention will be devoted to such spherical cells in this paper.

Viscosity

The term consistency is perhaps a better designation for the resistance of a cell interior to flow since it involves no implication as to whether the flow is truly viscous and proportional to the deforming force, or whether plastic, occurring only after a critical deforming force is reached. Experiment has shown that the cell interior is in some

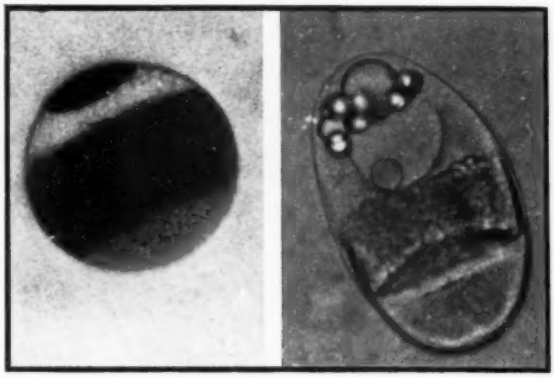


FIG. 3. Left—centrifuged sea urchin (Tripneustes) egg showing oil, clear, yolk and mitochondria layers. Right—a worm (Nereis) egg centrifuged in an air turbine at 84000× gravity, showing oil globules, a large nucleus with small nucleolus inside, a band of large yolk granules and very fine granules below.

cases a gel and in others quite liquid. It is also observed that a region near the surface of many cells is of greater consistency than the interior and is designated as the ectoplasm or cortical layer to distinguish it from the more liquid endoplasm. There are likewise special regions in the cell which may be jelled while others are not. Such gross differences as the above may be detected by the methods of microdissection and microinjection of material (oil or water droplets) whose behavior as observed with the microscope give evidence of the nature of the region concerned. Thus Chambers (1917)[14] has shown that the aster, whose appearance precedes the division of a cell into two, is a region in which jelled fibers grow out from a center and only the spaces between the fibers are liquid. The granules in the cell are pushed in lines between the fibers. In fixed and sectioned preparations of cells the granules appear to form rays and where two asters are present with a spindle between them the whole picture is like that of a field of force between unlike poles. Such an interpretation is tempting, but there is grave evidence against this view, most convincing the fact that when three asters appear in a cell the "lines of force" are such as would appear only if all three poles were unlike, an obviously impossible situation. How-

ever, the series of changes which we observe during cell division is entirely too complex to be considered here and we are very far from a rational interpretation of them. For many interesting observations on the behavior of specific structures in cells the reader is referred to the article by Chambers (1924)[2] in the *General Cytology* and to other books on cytology (Wilson, 1925;[10] Gray, 1931;[3] Sharp, 1934[9]).

It is quite obvious that the classical methods of measuring viscosity,—rate of flow through capillary tubes or resistance to rotation of a cylinder immersed in the fluid—cannot be used with living cells. We can, however, make use of the Einstein equation for mean displacement of a particle during Brownian movement or the Stokes' equation for movement of a particle in a gravitational or centrifugal field, both of which involve the viscosity of the medium. The movement of iron or nickel particles in a magnetic field has also been used (Heilbronn, 1922);[33] but the technical difficulties involving this method are very great.

According to Einstein (1905)[22] the mean displacement in direction x, $(\bar{D}_x)$ of a spherical

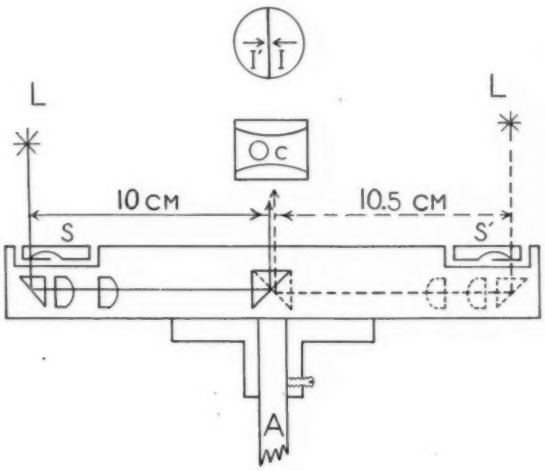


FIG. 4. Optical system for viewing two objects simultaneously with centrifuge-microscope. S, S', slides to hold material; L, L', light sources; A, axis of rotation; $Oc.$, stationary ocular.

particle, large in relation to the molecules of the medium, is given by:

$$\bar{D}_x = (RTt/3\pi N\eta r)^{\frac{1}{2}},$$

in which R is the gas constant, T the absolute

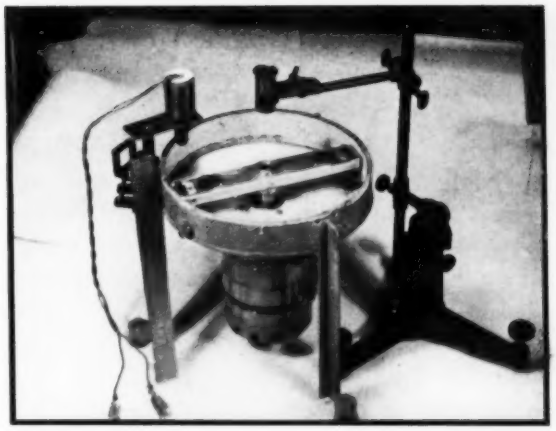


FIG. 5. One of the original models of the centrifuge microscope showing light (in tube with wires and clips), an auto headlight 6-volt bulb, the image of whose filament is thrown on the material to be examined by a low power microscope objective lens system. Note rotating bar containing lenses and prisms with its protecting ring, and the stationary ocular which can be raised and lowered for focusing.

temperature, N the Avogadro number, t, time, r, the radius of the particle and η, the viscosity of the medium. The relation was tested for gamboge particles by Perrin (1909)[42] who obtained good agreement with theory. Cells contain many small granules of the order 1μ in diameter whose displacement can be followed in many cases. Thus, Baas-Becking, Bakhuyzen and Hotelling (1928)[12] by an extensive statistical study of particles in Spirogyra, a fresh water alga, came to the conclusion that no one value could be given for the viscosity of the protoplasm of this cell but that it varied from place to place and from time to time. Maximum values were 47 and minimum values two times that of water, whose viscosity is one centipoise. Heilbrunn (1928)[5] obtained four centipoises for Arbacia, Harris (1935)[25] twenty centipoises for eggs of the worm, Sabellaria, and Pekarek[41] (1930–33), who has studied many cells, six centipoises for amoeba endoplasm.

Stokes' law has been widely used (Heilbrunn, 1928)[5] for determining relative viscosity of cells under different conditions and also for obtaining absolute viscosities, although the latter is subject to some uncertainty, since the cell is frequently filled with a large number of granular spheres of different sizes which move as a whole under the influence of gravity. Strictly, Stokes' law applies to a single spherical particle moving in a vessel of

sufficient size, so as to be unaffected by the walls or other particles, thus:

$$V = 2cg(\Delta\rho)r^2/9\eta,$$

in which V is velocity of fall, g the force of gravity, 980 dynes, C the centrifugal force in terms of gravity, $\Delta\rho$, the difference in density between granule and medium, r, the radius of the particle and η the viscosity. To allow for the influence of other particles falling at the same time, the Cunningham (1910)[17] correction must be applied. This reduces the apparent viscosity but there is considerable uncertainty in its estimation.

Marine eggs offer favorable material for investigation. When the eggs of the sea urchin, Arbacia punctulata, are centrifuged, the various granules separate by virtue of their different densities and form layers occupying spherical segments from which the volume of the materials present in an egg can be calculated. These figures are again approximate since it is not certain how much packing of the granules occur. If each layer consists of granules all of the same size (not the case) and just touching they would occupy about 75 percent of the segment. Allowing for this we find in Arbacia eggs one percent oil, 4.8 percent mitochondria, 27.2 percent yolk and 0.4 percent of nucleus suspended in 61.1 percent clear fluid whose viscosity is to be determined (Harvey, 1932)[26] as shown in Fig. 2. These pictures were taken through the centrifuge microscope while the eggs of Arbacia were being centrifuged and show successive movement of the granules into layers. Fig. 3 shows the distribution of materials in two other eggs, Tripneustes and Nereis.

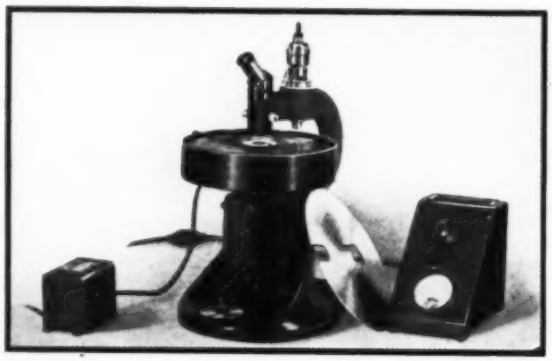


FIG. 6. Bausch and Lomb model of centrifuge microscope with transformer for light and speed control box.

In determining viscosity, the time for movement of granules through one-quarter or one-third the diameter of the egg is determined with a given centrifugal force, the radius and density of granule and medium is measured and the viscosity of the medium calculated. With different centrifugal forces between 5000 and 100 times gravity, the viscosity is the same provided the values are corrected for granule diffusion and streaming movements in the cell (Howard, 1932).[36] There is thus no evidence of plastic effects in this particular egg. Heilbrunn (1926)[34] obtained two centipoises for the Arbacia egg and four centipoises for the egg of a mollusk, Cumingia. His value for amoeba dubia was two centipoises (1929). Thus we are led to the conclusion that the viscosity of most cells is two to six times that of water, about equivalent to that of dilute portein solution. Some protein solutions, notably fibrinogen, which is responsible for the coagulation of blood, may show extraordinarly rapid viscosity changes at certain critical concentrations and it is known that cells also may behave in this way.

It must be remembered that the centrifuge as well as the Brownian movement methods measure the viscosity of the fluid of the cell in which a large number of granules are suspended. It is also possible to speak of the viscosity of the cell as a whole in the sense of the viscosity of a dense suspension of particles. Whole blood has a viscosity some four times that of the plasma which is left when the thirty-five percent of corpuscles are removed by centrifuging.

It is possible to give values for the cell as a whole only by applying such relations as those of Einstein (1906)[22] or Hatschek (1910).[32]

$$\eta_s = \eta_m(1+kf),$$

in which η_s is the viscosity of the suspension, η_m the viscosity of the medium, f the volume percent of particles and k a constant which Einstein places at 2.5 and Hatschek at 4.5. If there are forty percent of granules in the Arbacia egg the viscosity as a whole might be 6 to 9 centipoises as compared with three for the egg fluid. Other relations between the viscosity of a suspension and that of the suspending fluid have been proposed

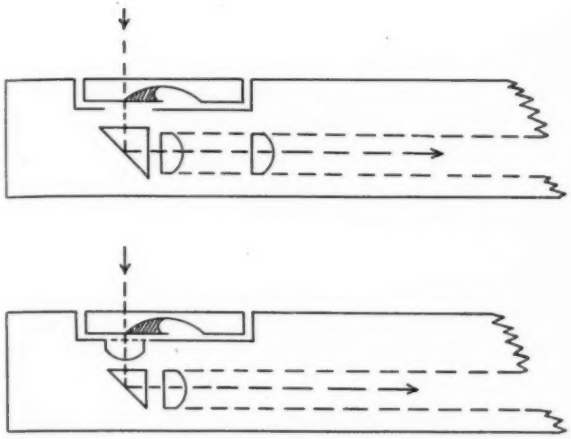


FIG. 7. Detail of objectives of centrifuge microscope showing arrangement of lenses and prism under slide for low power (above) and high power (below) magnification.

but the deviations are marked where the volume of suspended particles is large and their value is dubious when applied to the cell.

The change in viscosity of a cell under different conditions or in different regions is more important than absolute viscosities. Its determination would be greatly expedited if rate of movement of granules in a cell could be observed while they were being centrifuged. This can be done by means of the centrifuge-microscope, an instrument developed in collaboration with A. L. Loomis of the Loomis Laboratory, Tuxedo Park, New York. (Harvey and Loomis, 1930).[28] For study with this instrument the cells are suspended in a medium of the same density and placed in a special microscope slide so constructed that when centrifuged the cells are thrown exactly in position to be observed through an objective mounted on the centrifuge periphery. The image is brought to the center of rotation by a right angle prism and then directed up on the axis of rotation to be observed by an ocular which is stationary. Focusing is attained by moving the ocular or by interposing a movable lens between ocular and objective.

In the original design, a high tension electric discharge in mercury vapor under pressure, triggered off with each revolution of the centrifuge, was used as a source of light. This gives a perfect image over the whole field, but a sufficiently clear image can be obtained by a line of light, the image of a straight filament incandescent lamp parallel to a radius of rotation. It is focused on the object to be observed by a lens. With each revolution of the centrifuge the cells are illuminated by a narrow line of steady light and if observed directly the field would be blurred but when brought to the center of rotation the blurring is so reduced (i.e., a 0.1 mm movement at the periphery becomes a negligible movement at the center of rotation) that the image is clear and sharp. The microscope centrifuge differs radically from the optical system used for photographing the boundary of molecules in sedimentation constant determinations, since an image is formed, perfect in two planes, and not a line of demarcation in one plane.

The optical system is shown in Fig. 4 for a centrifuge microscope in which two groups of cells can be observed simultaneously, one the control and one the experimental material, for study of viscosity under different conditions (Harvey, 1933).[26]

Figure 5 shows one of the original instruments and Fig. 6 a commercial model manufactured by the Bausch and Lomb Optical Company. The

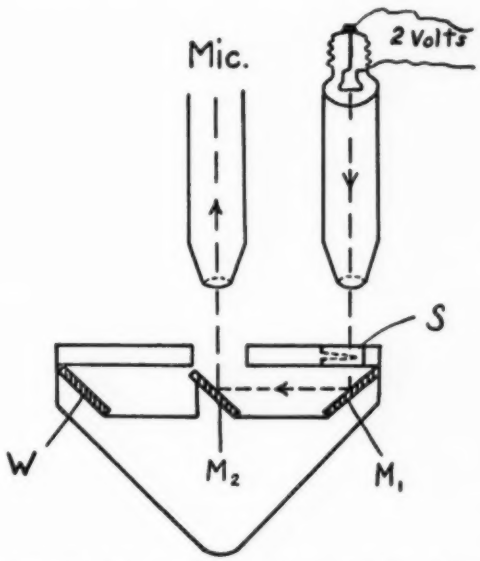


FIG. 8. Optical system for ultracentrifuge microscope. *S*, slide to hold material; M_1 M_2, metal mirrors; *W*, counterweight; *Mic.*, objective of compound microscope for viewing image on axis of rotation.

highest powers of the microscope can be used and the clearness of the image is independent of the rate of revolution. Fig. 7 shows the mounting of lenses and right angle prisms for high and low powers.

The principle can be applied to the Beams (1931)[13] air turbine (Harvey, 1932).[26] The optical system (Fig. 8) is simplified in this case to avoid rotation of glass lenses. Only metal mirrors are used to bring an image of the material under investigation to the center of rotation where it

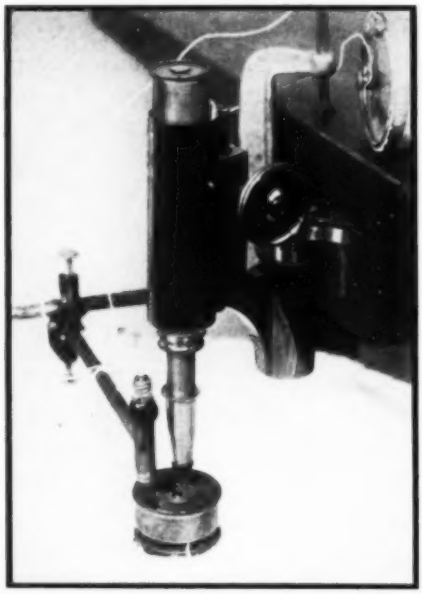


FIG. 9. Air turbine for ultracentrifuge microscope observation. Note rotor resting on stator and flashlight bulb illumination. Optical system as in Fig. 8.

is observed with a compound microscope which does not revolve. In this case the magnification is limited by the working distance of the objective but with a high power ocular 100× magnification can be easily attained. Fig. 9 shows the complete outfit. A modification of the optical system has been suggested by Pickels (1936)[43] in which a mirror is placed below the object and at an equal distance from object and center. If observed with a microscope from the side the virtual image will be on the axis of rotation and will appear to stand still.

When the movement of individual granules in cells is observed with the centrifuge microscope, it is found that many move uniformly but others, especially those moving down after the great bulk of granules has been displaced, frequently move and stop or move slowly then faster, as if they were caught by or stuck to a more gelled portion of the cell. This behavior was especially marked with amoeba (Harvey and Marsland, 1932)[30] and leads to the view that the cell is in a state of incipient gelation, i.e. the protoplasm is in such a condition that a slight alteration may change it from the sol to the gel state and vice versa.

Apart from the general value of knowing the physical constants for various cells, the figure for viscosity is important in our understanding of cell processes. Thus rapid sol⇌gel transformation in local regions can be observed particularly well in amoeba while moving and is believed to be the cause or at least intimately connected with amoeboid movement in general.

Cell division involves a cyclic series of changes in viscosity which have been carefully studied in the Arbacia egg (Heilbrunn, 1920;[34] Fry and Parks, 1934)[24] and in a mollusc egg, Cumingia (Heilbrunn, 1921).[34]

Stimulation of cells by various means has been observed to change the viscosity. Heilbrunn and Daugherty (1933)[35] find that stimulation (electric current or ultraviolet light) causes liquefaction of the cortical layer of amoeba dubia, followed by transient liquefaction and then gelation of the interior. The effect is reversible and apparently due to liberation of bound Ca from the cortex which changes the viscosity of the interior. The changes are part of a complex series of events taking place when the cell system is "stimulated." The stimulation may result in form changes in an amoeba, shortening of a muscle fiber, conduction of an impulse in nerve, luminescence in a photogenic cell, etc. Phenomena connected with stimulation of cells present one of the most fascinating fields of biophysics but space does not permit its consideration here.

Mechanical agitation of amoeba by shaking also changes the viscosity (after a preliminary decrease) in the direction of an increase (Angerer, 1936),[11] a phenomenon which is likewise believed to be connected with the liberation of Ca from a bound state. On the other hand agitation of the interior of marine eggs by microneedles at the time the gelled astral rays extend through the interior results in liquefaction and temporary disappearance of the rays, but they reappear again if the churning is not continued (Chambers,

1917).[14] This effect may be compared to that observed with certain types of inorganic gels [$Fe(OH)_3$] referred to as thixotropy (Freundlich, 1927).[23] In general the changes in cells during stimulation are in the same direction as those connected with death, when the material of most cells sets to a firm gel. Thus, eggs

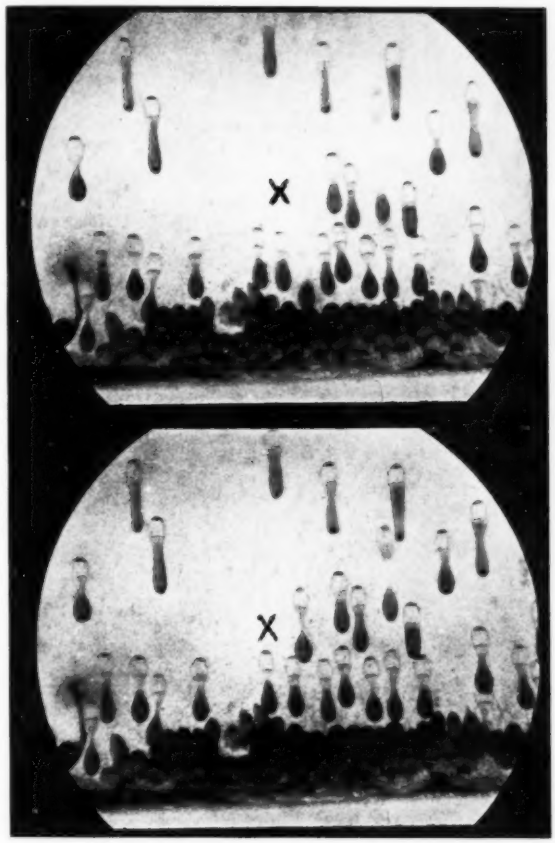


FIG. 10. Two frames of a moving picture film taken through the centrifuge microscope showing sea urchin eggs pulling into two halves by centrifugal force. (5500× gravity). Note that the egg to right of cross in lower picture has separated and disappeared in upper picture. Frames 2 seconds apart; 0.8 second exposure; revolving 7500 r.p.m.

squeezed through a capillary tube of slightly smaller diameter than the egg quickly round up but if the tube is very small so that undue elongation of the egg occurs, they remain as gelled cylinders. Such eggs do not develop when fertilized.

Temperature has a marked effect on viscosity. The general change is decrease with increasing temperature but frequently a maximum viscosity is found for a certain temperature (7° in amoeba proteus, Thornton, 1935[47]) below which decrease in viscosity again occurs.

The effect of anaesthetics, salts, and changes in the H ion concentration have also been investigated but space does not allow the results to be given. Such studies are particularly important in the analysis of physiological processes which are specifically affected by the agents enumerated.

Tension at the Surface

For tension studies, only cells can be used which lack rigid protecting membranes or a gelled surface layer or the determination must be made after the protecting coats are removed. Although the word "plasma membrane" is used for the external surface of a cell, which is responsible for its osmotic behavior, much evidence is accumulating to indicate that this surface layer is of molecular thickness. It is probably not correct to speak of the surface tension of a cell in the sense of an interfacial tension between two pure nonmiscable liquids but a definite tension can be measured, which represents the sum of surface and elastic forces best designated as "tension at the surface."

Indeed, the whole question of a membrane becomes a matter of definition and all gradations of surface films exist from the monolayer through multilayers to molecular structures sufficiently thick to be unequivocally designated a membrane. When it is recalled that even monolayers may exhibit the properties of two dimensional gases, liquids and solids with all degrees of two dimensional viscosity and elasticity (Langmuir, 1936)[38] the complex situation in regard to the cell becomes obvious.

No one would deny that a soap bubble in air can be said to possess surface tension but its value will depend on the bulk concentration of soap. An albumen bubble also possesses a surface tension which depends not only on the bulk concentration of albumen but also on the area of its surface, i.e., it possesses elastic properties, with increased tension for greater areas, and in addition shows hysteresis (Harvey and Danielli, 1936).[29] The values for tension of albumen bubbles depend on whether the tension (calculated from the simple relation $P=4T/r$,

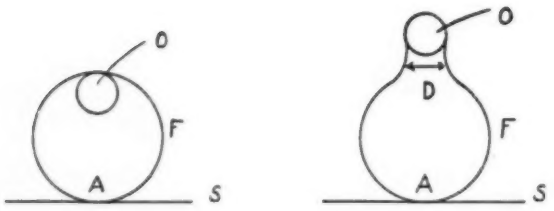


FIG. 11. Diagram of a drop of oil (O) pulling out of cell (A) resting on surface (S) in a fluid (F). The system becomes unstable when a neck of the cell is pulled out whose diameter (D) equals that of the oil drop.

where P = pressure, T = tension, and r = radius of the bubble) is determined as the bubble is expanded or as it is contracted.

Actually the egg of Arbacia is surrounded by a jelly and also by a "pellicle" which is so intimate a part of the surface that it merges with the protoplasm. The jelly can be removed by shaking and the pellicle by microdissection or washing the eggs with KCl. It is a viscid liquid that can be centrifuged toward one end of the cell. When the egg is fertilized the pellicle is lifted away from the surface and hardens to become the fertilization membrane, a rigid structure. Even with a pellicle present the eggs behave like viscous liquid drops and can be treated as such in determining their tension. Other "naked" cells which are suitable for tension studies are amoeba, leucocytes and plant cells removed from their cellulose walls.

One would like to insert a micromanometer into a spherical cell and determine the tension from the relation, $P = 2T/r$, but technical difficulties prevent this method. It is also obvious that such classical methods of determining surface tension as the capillary rise or adhering rings or drop weight cannot be used with cells, but several independent methods available all show that—(1) the tension, i.e., the sum of surface and elastic tensions is low, less than 1 dyne/cm, (2) the surface has elastic properties.

The Centrifuge Method

This assumes that a spherical cell behaves like a fluid droplet and becomes unstable when drawn into a cylinder of the same volume whose length is π times its diameter, breaking into two or more spheres. It is applicable to egg cells containing oil globules (lighter than the cell fluid) and yolk granules (heavier than the cell fluid). Under the influence of centrifugal force the oil and yolk separate and exert stretching forces that pull the cell into two almost equal spheres (Harvey, 1931).[26] Fig. 10 shows two frames of a moving picture film in which Arbacia eggs are pulled into two. It will be noted that the egg to right of the cross in the lower picture has disappeared in the upper. At the moment of instability we may equate these forces to the tension (T) around the circumference of the elongated cylinder, thus:

$$\pi DT = Cg[V_H(\rho_H - \rho_M) + V_L(\rho_M - \rho_L)],$$

where D = diameter of cylinder, C = centrifugal force in times gravity, $g = 980$, V_H = volume of

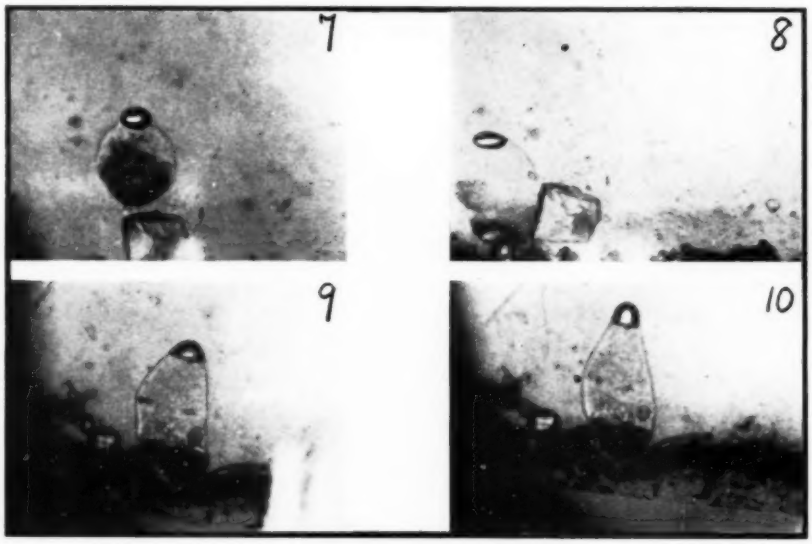


FIG. 12. Photographs of amoeba proteus (7, 8) and amoeba dubia (9, 10) containing injected oil drops, photographed while revolving in the centrifuge microscope. Note firm surface of A proteus, against which the oil flattens and the pulling out of A dubia by the buoyant oil which will, with higher forces, pull away from the amoeba completely. The centrifugal force in 7 is 5300; in 8, 12500; in 9, 4800 and in 10, 5750 times gravity.

heavy half, ρ_H = density of heavy half, ρ_M = density of medium, V_L = volume of light half and ρ_L = density of light half.

The whole process of separation into two half-cells can be watched (or photographed) in the centrifuge-microscope and the centrifugal force that is just sufficient to separate the two halves determined. Volumes and densities are easily measured. Results for the unfertilized eggs of a sea urchin, Arbacia punctulata, range around 0.2 dynes/cm depending on the individual from which eggs are obtained and the time they remain standing in sea water, for the tension increases with time. The surface of such an unstable cylinder would be about 25 percent greater than the surface of the original sphere so that the value 0.2 dyne/cm represents a 25 percent increase of the surface.

A modification of this method can be applied to cells into which oil drops have been injected. In this case the oil drop is pulled out by centrifugal force and the figure becomes unstable when a neck of protoplasm forms equal to the diameter of the oil drop (Fig. 11). We equate the buoyant force of the oil when centrifuged to the tension around the circumference of the neck of protoplasm when the oil drop pulls away:

$$\pi DT = Cg[V_0(\rho_M - \rho_0)],$$

where D = diameter of neck, V_0 = volume of oil drop, ρ_0 = density of oil, ρ_M = density of medium surrounding cell.

Values obtained by this method for amoeba dubia (see Fig. 12) are 1 to 3 dynes/cm (Harvey and Marsland, 1932);[30] for a slime mould, Physarum polycephalum, around 0.45 dyne/cm (Vexler, 1935);[48] and for rabbit macrophages 2 dynes/cm; frog leucocytes, 1.3 dynes/cm (Shapiro and Harvey, 1936).[44]

Compression Method

By the use of the general equation:

$$F/A = P = T(1/R_1 + 1/R_2),$$

and by measuring the force (F) necessary to flatten a cell a given amount, as illustrated in Fig. 13, Cole (1932)[15] has not only determined the tension of the unflattened egg but obtained values for the increase in tension as the surface area increased on flattening. The eggs are flattened by a microbeam of gold, 6μ thick and 180μ wide, pressing on the top of the egg, and then photographed. The two radii of curvature (R_1, R_2) can be measured from the photographs

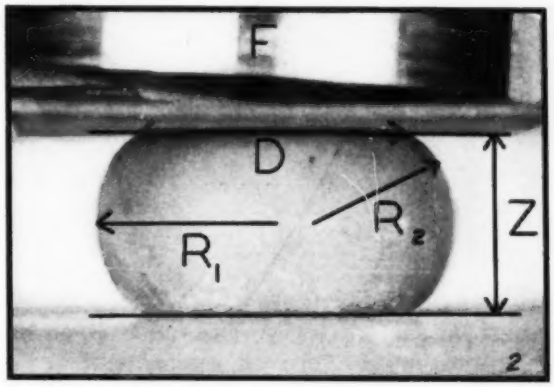


FIG. 13. A sphere flattened between two planes by a force F, showing measurements to be made in calculating the tension at the surface.

and the pressure (P) calculated from F/A, where A is the area of the egg flattened by contact with the beam surface. The beam, held in such a manner that it always remains parallel to the plane of compression, is calibrated by hanging microweights on one end and noting its deflection by light reflected from the beam to a scale. The forces to be measured are of the order of 10^{-6} grams, 2 micrograms giving a compression of 25μ.

Results on the unfertilized egg of the sea urchin, Arbacia punctulata, 74μ in diameter give a tension of 0.13 dyne/cm when compressed 25μ, and lower values for less compression. Extrapolation of the tension-compression curve, which is slightly convex to the compression axis, to zero compression, gives a value of 0.08 dynes/cm for the uncompressed egg. The evidence for elasticity of the surface is clear and conclusive. The fertilized egg of Arbacia (Cole and Michaelis, 1932)[16] without fertilization membranes behaves as the unfertilized and gives the same low values for surface forces until shortly before first cleavage but the fertilized eggs with fertilization membranes behave as if the membrane were decidedly rigid and the values have no significance.

Kinetic Method

Just before the completion of first cleavage of an Arbacia egg the two blastomeres are connected by a small stalk. If one blastomere is punctured, the remaining one will discharge its contents through the stalk due to an excess internal pressure from the tension at the surface. From moving pictures, the rate of discharge can be determined by measuring the decrease in volume of the blastomere. It follows a law which would indicate elastic forces at the surface. Assuming Poisseuille's law (and using a measured value for viscosity of the egg fluid at this stage of development), Sichel and Burton (1936)[45] calculated the excess internal pressure to be 62 dynes/cm² and the tension 0.09 dynes/cm, agreeing well with the Harvey (1931)[26] and Cole (1932)[15] figures for the same egg.

Sessile Drop Method

The various equations relating the form of a flattened drop resting on a plane to the tension at its surface have been summarized and discussed by Dorsey (1926, 1928, 1929).[21] In general the relation

$$T = g(d - d')r^2F$$

holds. T is the tension; g, the force of gravity, $d-d'$, the difference in density between drop and medium; r, the radius of greatest flattening and F a function containing f, a term representing the flattening of the drop.

$$F = 0.052\ f - 0.12268 + 0.0481f$$

and $$f = CB/r - 0.41421.$$

CB is defined with reference to Fig. 14.

Shapiro (Harvey and Shapiro, 1934)[31] has given a table relating F and f for different degrees of flattening. These equations strictly apply only where pure surface tension is involved, i.e., only where the tension is independent of the extension of the surface. It is easy to calculate that most marine eggs are far too small to flatten appreciably under the influence of gravity even though their surface tension is assumed to be 0.2 dynes/cm (Harvey, 1933).[26]

The egg of the mollusc, Busycon canaliculatum, 1 mm in diam. does flatten under the influence of gravity and gives a tension of 0.5 dynes/cm while the egg of the salamander, Triturus viridescens, 1.5 mm in diameter, gives 0.1 dyne/cm. The protecting membranes were of course first removed (Harvey and Fankhauser, 1933).[27] Both eggs cleave completely so that any membrane at their surface must be nonrigid if not completely liquid in character. The sessile drop equations do not apply to cells with membranes (Harvey, 1936).[26]

The most important application of the sessile drop equations is to small oil drops in living cells, made possible by the development of the centrifuge-microscope. Mackerel eggs contain a single droplet, 310μ in diameter, which flattens against the rigid cell membrane if the eggs are

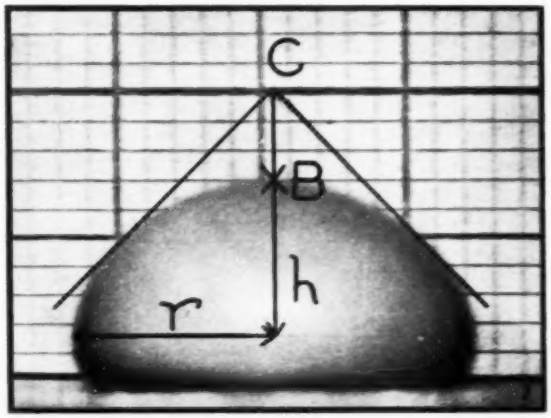


FIG. 14. A sphere flattened under the influence of gravity showing measurements to be made in calculating the tension at its surface.

centrifuged. If photographed while revolving beautiful sharp profiles of flattened drops are obtained as shown in Fig. 15 from which Harvey and Shapiro (1934)[31] calculated the oil-protoplasm interfacial tension to average 0.6 dynes/cm. When the force was increased from 50 to 450 times gravity little change in the tension occurred showing that the surface did not possess marked elastic properties.

Danielli and Harvey (1935)[20] studied the interfacial tension of egg oil extracted from mackerel eggs, against various aqueous solutions, including egg extract, by means of the du Nouy tensimeter. It was found that low tensions (0.8 dynes/cm) were obtained with oil in contact with egg extract but much higher ones with egg oil in contact with

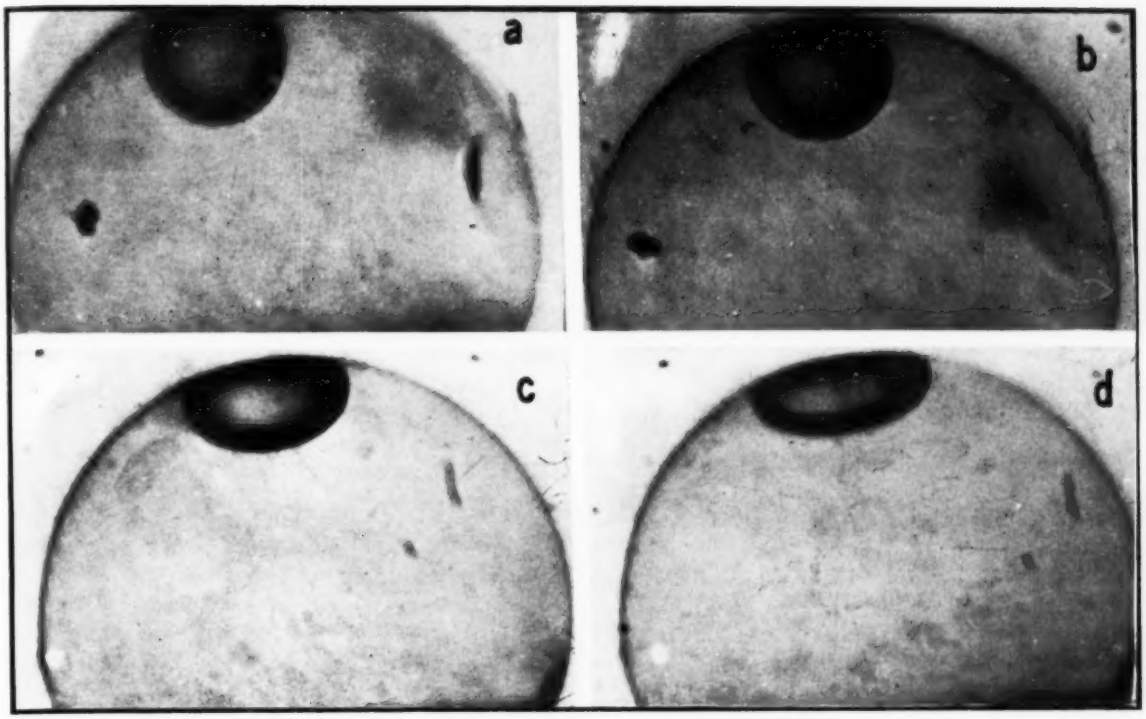


FIG. 15. A globule of oil in a fish egg photographed through the centrifuge microscope at 7 (*a*), 62 (*b*) 195 (*c*) and 400 (*d*) times gravity. Note the progressive flattening as the centrifugal force is increased. Values for the interfacial tension between oil and protoplasm average 0.6 dynes/cm.

sea water (7 dynes/cm). Further analysis of the cause of the low tension pointed to a globulin-like protein as the surface active substance. We can thus picture the oil drop surface as made up of oriented oil molecules, the polar groups toward the water phase, covered with an adsorbed monolayer of hydrated protein molecules. Since the tension at the surface of cells is correspondingly low, perhaps the same picture can be formed of the cell surface, a thin oil film with adsorbed protein on either side. This thesis has been elaborated by Danielli and Dawson (1935)[19] and by Danielli (1936),[18] who discuss properties of various types of lipoid films and come to the conclusion that a continuous lipoid film with adsorbed protein would be the most stable but that mosaic protein and lipoid films cannot be excluded on the basis of mechanical properties.

The lipoid theory of the cell surface has received support from permeability studies since the days of Overton (1895)[40] who first suggested it and pointed out that fat soluble substances penetrated cells with ease and in proportion to their lipoid solubility. It is also supported by the studies on alternating current resistance of cells, where impedance measurements have led to values for surface capacity of the order of one microfarad per cm^2. Although the interpretation of impedance data is very complex (see Cole, 1937)[15] the cell surface behaves in part like a condenser whose dielectric *might* be lipoid and of molecular thickness.

Finally the work of Kopac and Chambers (1937)[37] has shown that living cells will coalesce with oil. Clean oil drops touched to the surfaces of washed Arbacia eggs pass into the cell quickly, thus changing from system *A* (cell and oil in water) to system *B* (oil in cell in water). The conditions for coalescence of liquid spheres have been discussed by Taylor (1921).[46] By applying these it can be shown that the energy of system *A* is greater than that of system *B* and consequently the change to *B* should occur spontaneously provided the surface is liquid. That is what we observe to happen—the oil is engulfed. For these

calculations of surface energy the area of the spheres and the previously mentioned values for the Arbacia egg-sea water and oil-protoplasm tension together with measured values for oil-sea water interfacial tension are used.

It is experiments such as I have outlined in this review, which, combined with the work of Langmuir, Rideal, Adam and Harkins on mono- and multimolecular layers, will open up a rich field for the biophysicist in his exploration of the functioning of a cell.

Selected Bibliography

Books

1. N. K. Adam, *Physics and Chemistry of Surfaces* (Oxford, 1930).
2. R. Chambers, *The Physical Structure of Protoplasm in General Cytology*, edited by E. V. Cowdry (Chicago, 1924).
3. J. Gray, *Textbook of Experimental Cytology* (Cambridge, 1931).
4. W. D. Harkins, *Surface Energy and Surface Tension in Colloid Chemistry*, edited by J. Alexander, Vol. I (New York, 1926).
5. L. V. Heilbrunn, *The Colloid Chemistry of Protoplasm* (Berlin, 1928).
6. R. Hoeber, *Physikalische Chemie der Zelle and Gewebe*.
7. E. K. Rideal, *Surface Chemistry* (Cambridge, 1930).
8. W. Seifritz, *Protoplasm* (New York, 1936).
9. L. Sharp, *Introduction to Cytology* (New York, 1934).
10. E. B. Wilson, *The Cell in Development and Heredity*, third edition (New York, 1925).

Papers

11. C. A. Angerer, J. Cell. Comp. Physiol. **8**, 329 (1936).
12. L. G. M. Baas-Becking, H. Bakhuyzen, and H. Hotelling, Verh. Akad. Wetensch. Amsterdam **25**, 5 (1928).
13. J. W. Beams, Science **74**, 44 (1931); Rev. Sci. Inst. **1**, 667 (1930).
14. R. Chambers, J. Exp. Zool. **23**, 483 (1917); J. Gen. Physiol. **2**, 49 (1919); **4**, 33, 41 (1921).
15. K. S. Cole, Trans. Faraday Soc. "Discussion on Membranes," 966 (1937); J. Cell. Comp. Physiol. **1**, 1 (1932).
16. K. S. Cole and Eva Michaelis, J. Cell. Comp. Physiol. **2**, 121 (1932).
17. Cunningham, Proc. Roy. Soc. **83A**, 357 (1910).
18. J. F. Danielli, J. Cell. Comp. Physiol. **7**, 393 (1936).
19. J. F. Danielli and H. Dawson, J. Cell. Comp. Physiol. **5**, 495 (1935).
20. J. F. Danielli and E. N. Harvey, J. Cell. Comp. Physiol. **5**, 483 (1935).
21. W. E. Dorsey, Sci. Papers Nat. Bur. Standards, **21**, 563 (1926); J. Wash. Acad. Sci. **18**, 505 (1928); Bull. Nat. Res. Coun., No. 69, p. 56 (1929).
22. A. Einstein, Ann. d. Physik. **17**, 549 (1905); **19**, 289 (1906); **34**, 591 (1911); Zeit. f. Elektrochem. **14**, 235 (1908).
23. H. Freundlich, Protoplasma **2**, 278 (1927).
24. H. J. Fry and M. C. Parks, Protoplasma **21**, 473 (1934).
25. J. E. Harris, J. Exp. Biol. **12**, 65 (1935).
26. E. N. Harvey, Biol. Bull. **61**, 273 (1931); **62**, 141 (1932); J. Cell. Comp. Physiol. **4**, 35 (1933); **8**, 251 (1936); J. Frank. Inst. **214**, 1 (1932); Science **75**, 267 (1932); **77**, 430 (1933).
27. E. N. Harvey and G. Fankhauser, J. Cell. Comp. Physiol. **3**, 453 (1933).
28. E. N. Harvey and A. L. Loomis, Science **75**, 42 (1930).
29. E. N. Harvey and J. F. Danielli, J. Cell. Comp. Physiol. **8**, 31 (1936).
30. E. N. Harvey and D. A. Marsland, J. Cell. Comp. Physiol. **2**, 75 (1932).
31. E. N. Harvey and H. Shapiro, J. Cell. Comp. Physiol. **5**, 255 (1934).
32. E. Hatschek, Kolloid Zeits. **7**, 301 (1910).
33. A. L. Heilbronn, Jahrb. wiss. Bot. **61**, 284 (1922).
34. L. V. Heilbrunn, J. Exp. Zool. **44**, 255 (1926); **34**, 417 (1921); **30**, 211 (1920); Protoplasma **8**, 65 (1929).
35. L. V. Heilbrunn and K. Daugherty, Protoplasma **18**, 596 (1933).
36. E. Howard, J. Cell. Comp. Physiol. **1**, 355 (1932).
37. M. J. Kopac and R. Chambers, J. Cell. Comp. Physiol. **9**, 331, 345 (1937).
38. I. Langmuir, J. Frank. Inst. **218**, 143 (1934); Science **84**, 379 (1936); **85**, 76 (1937).
39. J. C. Maxwell, *Treatise on Electricity and Magnetism* (Oxford, 1873).
40. E. Overton, Vierteljahrs. Naturforsch. Ger. Zurich **40**, 159 (1895); **41**, 383 (1896); **44**, 88 (1899).
41. J. Pekarek, Protoplasma **10**, 510 (1930); **11**, 19 (1930); **13**, 637 (1931); **17**, 1 (1933); **18**, 1 (1933); **20**, 251, 359 (1933).
42. J. Perrin, Ann. Chim. et Phys. **18**, 4 (1909).
43. E. G. Pickels, Science **83**, 471 (1936).
44. H. Shapiro and E. N. Harvey, J. Cell. Comp. Physiol. **8**, 21 (1936).
45. F. J. M. Sichel and A. C. Burton, Biol. Bull. **71**, 397 (1936).
46. W. Taylor, Phil. Mag. **41**, 877 (1921).
47. F. E. Thornton, Physiol. Zool. **8**, 246 (1935).
48. D. Vexler, Proc. Soc. Exp. Biol. and Med. **32**, 1539 (1935).

Diffusion Processes in Living Systems*

By M. H. JACOBS
University of Pennsylvania
Philadelphia, Pennsylvania

IN every cell of every plant and animal at every instant of time diffusion processes are in progress, bringing into the organism necessary materials such as dissolved foodstuffs and oxygen, distributing these materials in a useful manner, and removing waste substances that would otherwise be harmful. Without diffusion life would be impossible.

And yet this situation presents a curious paradox. Diffusion processes in their last analysis are diametrically opposed to all that is most characteristic of life. By nature they are essentially destructive. In accordance with the second law of thermodynamics they are continually engaged in increasing the disorder or randomness of the universe by transferring matter from less probable to more probable distributions. But organisms, as their name indicates, are characterized by organization, that is, by a high degree of improbability of arrangement. This they not only preserve, but they tend to increase during the development of the individual and the history of the race. Diffusion processes if unchecked, as they largely are after death, would quickly put an end to such an incredibly improbable arrangement of matter as the body of even the simplest organism. But during life they not only fail to destroy this characteristic state of organization, but they are necessary for its preservation. How living beings have succeeded in harnessing, so to speak, these fundamentally destructive processes and in using them for constructive purposes is one of the major mysteries of biology.

It would obviously be impossible in the time at my disposal to enter very far into a discussion of a situation of such complexity, even if the necessary knowledge were available, which is far from being the case. It will therefore be more profitable to consider instead certain peculiarities of diffusion in a single structurally very simple type of cell, the vertebrate red blood corpuscle or erythrocyte. The advantages of the erythrocyte for this purpose are that in it conditions for diffusion are reduced almost, though fortunately not quite, to their lowest terms. It therefore enables us to consider complicating factors one at a time without undue confusion, and serves as a convenient stepping stone between the simplest diffusion processes in nonliving systems and the incredibly complex conditions encountered in most living cells.

The erythrocyte in the adult mammal begins its existence in the bone marrow as an entirely typical cell which grows and divides in the ordinary way. At this early stage it presents all the unsolved problems of diffusion encountered in other actively developing cells. But during its life history it changes its nature and becomes more and more simplified, losing its nucleus and other visible internal structures, its powers of repairing injuries, and most of its metabolism. In its mature condition we may think of it—though with certain reservations—as consisting essentially of a solution of hemoglobin and of certain salts and organic compounds surrounded by a film or membrane of very peculiar properties.

This membrane, which so profoundly modifies diffusion processes in the erythrocyte as well as in other cells, is too thin to be studied by direct

* Presented at the Symposium on Biophysics, Philadelphia, Pennsylvania, November 4, 5 and 6, 1937.

microscopic observation. Attempts to estimate its thickness have been made by Fricke[1] and by McClendon[2] by studying the electrical impedance of suspensions of erythrocytes. By making assumptions of somewhat doubtful validity they have arrived at estimated thicknesses of the order of magnitude of unimolecular films of, for example, fatty acids. Gorter and Grendel[3] on different grounds, which seem to be even more questionable, have postulated a bimolecular film. The work of others points to a structure of still greater complexity. Whatever the truth may ultimately prove to be, however, it is very likely that the thickness of the plasma membrane of the erythrocyte, like that of the artificial films to be described by Dr. Langmuir in the Johnson Foundation Lectures, is of molecular dimensions.

Many of the properties of this membrane can be investigated by studying its permeability to different types of dissolved substances. From the large mass of data now available in this field three rough generalizations may be made. The first of these is that substances which are sufficiently soluble in fat-like or lipoid substances, or in organic liquids which dissolve these substances, will enter the erythrocyte readily regardless of their molecular volumes—sometimes even more rapidly the larger the volume. A good example of this principle is furnished by glycerol and its mono- and diacetic acid esters, in which lipoid solubility increases with molecular weight and with it, usually, ability to penetrate living cells. Comparable amounts of these three substances can be shown to enter the beef erythrocyte at 20°C in times of the order of magnitude of 45 minutes, 50 seconds and 6 seconds, respectively. Their rates of diffusion in water, however, or even across the water-soaked artificial membranes studied by Collander,[4] decrease instead of increasing with the molecular weight.

So general is this behavior of lipoid-soluble substances that Overton was led many years ago to postulate a lipoid film at the surface of all cells. Whatever objections may be raised to the Overton theory in its original form, there is independent evidence of the importance of lipoids at the surface of the erythrocyte—for example the observation of Mudd and Mudd[5] that these cells when brought into an interface between water and an oil go into the oil, though certain other cells go into the water.

The factor of lipoid solubility, however, must not be allowed to overshadow other known facts which suggest a second generalization, namely, that substances whose molecular volumes are sufficiently small will enter the erythrocyte regardless of their lipoid solubility. In a group of *chemically related substances* of low lipoid solubility this rate is nearly always greater the smaller the molecule. A good example of this principle is furnished by the behavior of the homologous series of polyhydric alcohols having 2, 3, 4 and 6 carbon atoms, respectively, with the erythrocytes of several species of mammals.

In Table I are shown the average times ob-

TABLE I. *Average times in seconds for 75% hemolysis in 0.3 m solutions of polyhydric alcohols at 20°C. The symbol ∝ indicates that hemolysis occurs only after many hours or not at all.*

Species	Ethylene glycol CH_2OH CH_2OH	Glycerol CH_2OH $CHOH$ CH_2OH	Erythritol CH_2OH $(CHOH)_2$ CH_2OH	Mannitol CH_2OH $(CHOH)_4$ CH_2OH
Ox	27	2169	∝	∝
Sheep	24	1397	∝	∝
Dog	13	1272	∝	∝
Cat	13	1135	∝	∝
Guinea pig	5	188	∝	∝
Rabbit	5	51	∝	∝
Albino rat	4	18	½ to several hrs.	∝
Albino mouse	6	45	309	4620
White-footed mouse	4	9	28	597
Groundhog	1.9	2.2	6	80
Man	4	32	∝	∝

tained in one set of experiments for the production of the same degree of osmotic hemolysis at 20°C in approximately isosmotic solutions of the four substances in question, with the blood of 11 species of mammals. These times may with certain qualifications be taken as a measure of the relative rates of entrance of the various solutes into the cells. It will be noted that while there are striking differences in absolute permeability from species to species, the rate of penetration of the four substances in question for any given species decreases with the size of the molecule. Furthermore, the decrease in permeability from substance to substance is enormously greater than that of the rate of free diffusion, which for ordinary dissolved sub-

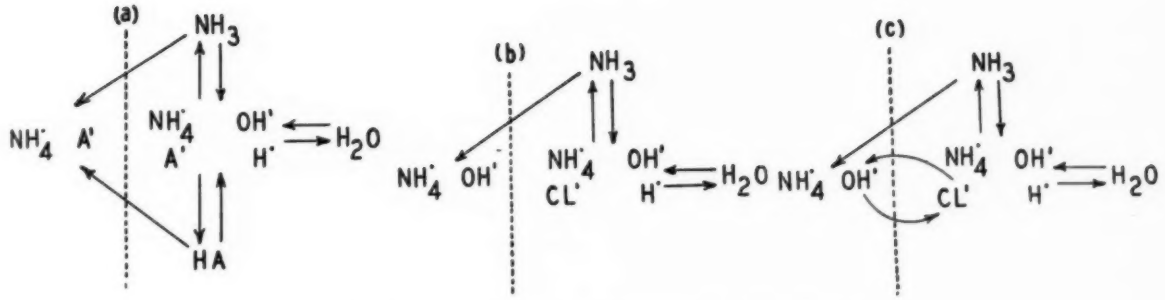


FIG. 1. Manner of entrance of ammonium salts into living cells.

stances varies roughly inversely with the square root of the molecular weight. The fact that the erythrocyte in this respect resembles certain artificial membranes which are known to have a porous structure suggests that its plasma membrane—and incidentally that of many other cells—may possess a certain degree of "porosity."

To these two generalizations, which apply more or less universally to living cells, a third may be added which is peculiar to the erythrocyte. It is that the plasma membrane of this cell under normal conditions is freely permeable to the anions of most inorganic electrolytes, but is impermeable to their cations. This peculiarity plays an important role in the transport of carbon dioxide by the blood, and is to be looked upon as a special adaptation of the red blood cell to the functions it has to perform in the body.

The permeability of the erythrocyte to anions explains a fact that was at one time puzzling, namely, that whereas it and all other known cells are rather freely permeable to the ammonium salts of most weak acids, it alone seems to be fairly permeable to ammonium salts of the strong acids such as NH_4Cl. In the case of ammonium acetate, what enters the cell seems to be not the ammonium and acetate ions, but rather the undissociated molecules of ammonia and of the free acid which are present through hydrolysis of the salt and which readily recombine within the cell (Fig. 1(a)).[6] In the case of ammonium chloride, however, the hydrochloric acid resulting from hydrolysis is practically completely ionized. Since ordinary cells are rather impermeable to ions in general, and especially so to anions, in a solution of NH_4Cl only free ammonia can enter them with any rapidity, leading to an increase in internal alkalinity, even though the external solution be acid in reaction (Fig. 1(b)). In the anion-permeable erythrocyte, on the other hand, the process does not end here, but an exchange of OH′ for Cl′ ions permits the entire salt to gain access to the cell (Fig. 1(c)).

These three peculiarities of the erythrocyte, having to do with lipoid solubility, molecular size, and differential permeability to ions may all be imitated by artificial models. As far as I am aware, all three characteristics have not yet been combined in a single model, but there would seem to be no insuperable obstacles to doing so. However, before accepting such a model as a satisfactory representation of the surface of an erythrocyte certain additional facts must be taken into account.

The first of these is that the plasma membrane of the red blood cell shows highly characteristic differences from species to species of animal. These differences are so constant and so striking that species may readily be separated from one another by the ease with which their erythrocytes admit certain substances. Thus, human blood can be distinguished from the other kinds commonly used in the laboratory by the permeability of its erythrocytes to glucose and certain other sugars; blood of the mouse by permeability to the tetrahydric alcohol, erythritol, and so on. The albino mouse and the albino rat are two animals which are very closely related—in fact zoologically they are two species of the same genus. And yet we now know at least a dozen ways based on specific permeability reactions by which their blood can readily be distinguished. The erythritol method, just mentioned, is one of these.

In cases of this sort the chief factor involved seems to be not the lipoid solubility or the size of the penetrating molecule, but, frequently at least, its chemical nature. Thus, as has been pointed out by Ulrich,[7] the human erythrocyte shows a more or less specific permeability to certain sugars, but a relative impermeability to polyhydric alcohols even of much lower molecular volume. Erythrocytes of the mouse, on the other hand, show little permeability to sugars but a relatively high degree of permeability to polyhydric alcohols. It may be noted in this connection that the erythrocytes of the wild white-footed mouse, Peromyscus, and especially those of the groundhog[8] combine both types of permeability.

By characters such as these it is easy to construct an analytical key, similar to those used by systematic botanists and zoologists, by which it is possible to identify the blood of some 20 species of mammals. The details of such a key are of no particular interest; what is of interest is the fact that in the case of the first 20 mammals that have received careful study there has been no particular difficulty in finding characteristic distinguishing features by the use of only a small number of penetrating substances. I see no reason to believe that with sufficient patience similar distinctions could not be found between most or all of the 35,000 known species of vertebrates. The problem of the nature of the cell surface is therefore enormously complicated by the necessity of explaining these and other as yet unrecognized specific differences. From a practical standpoint, however, the situation is not without its advantages, since in testing some given hypothesis of cell permeability it is very convenient to be able, by a proper choice of species, to secure erythrocytes having almost any desired combination of properties.

Another line of attack on the nature of the plasma membrane consists in a study of the kind of agents that modify its permeability. One case of this sort of some physiological interest has to do with the influence on the permeability of the plasma membrane of the so-called indifferent narcotics, of which *n*-butyl alcohol may here be taken as typical. One of the classical theories of the nature of narcosis is that it causes a reversible decrease in the permeability of the plasma membrane. Looking for this effect in the erythrocyte, we obtained indications of a much more complex situation than had previously been suspected. For example, with glycerol as the penetrating substance, it turned out the *n*-butyl alcohol in nonhemolytic concentrations—i.e., 0.25 M or less—greatly decreases the permeability of the erythrocytes of man, as well as those of the rat, mouse, rabbit, guinea pig, groundhog and some other species. This is in accordance with the classical theory. But in the case of the erythrocytes of the ox, sheep, pig, horse, dog, and cat permeability to glycerol is increased instead of being decreased.

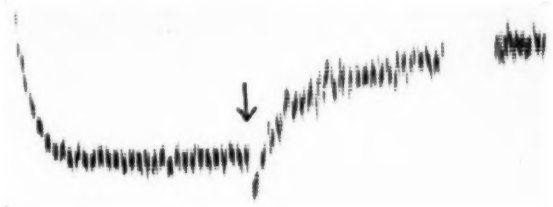


FIG. 2. Volume changes of beef erythrocytes first equilibrated with a 0.3 M sucrose solution, to which NaCl is added at the point indicated by the arrow.

It happens that the erythrocytes of the first group of animals are normally very permeable to glycerol, while those of the second group are very much less so. The effect of the alcohol, therefore, is to make all the erythrocytes more or less alike, which is what might be expected if it in some way entered into the composition of the cell surface. That the alcohol may perhaps act by blocking pathways normally open to the diffusing molecules is suggested by the fact that its effectiveness increases with the molecular volume of the latter. Thus with erythrocytes of the ground hog permeability to ethylene glycol is little affected by butyl alcohol; that to glycerol and erythritol is greatly diminished; and that to mannitol is practically abolished.

Since butyl alcohol is itself a lipoid solvent, it is not surprising to find that it seems typically not to decrease but rather to increase the permeability of erythrocytes to lipoid-soluble substances. This was found to be true with a variety of substances and species of animals. The behavior of monoacetin in this respect may be taken as typical; for some reason thiourea,

though not very lipoid-soluble, also behaves in the same way.

On the other hand, in all the species so far studied without exception, permeability to ammonium salts of the mineral acids is always decreased by butyl alcohol. This probably indicates a general decrease in the permeability of the cell to anions—an effect of which we have other independent evidence. Permeability to ammonium salts of the type of the acetate, on the other hand, in which penetration is believed to occur in a molecular rather than in an ionic form (see Fig. 1(a)), as might be expected, proves to be increased rather than decreased by butyl alcohol.

We have, therefore, a somewhat complicated set of facts. With some substances butyl alcohol increases permeability in all the species so far studied; with others it decreases permeability in the same species; with still others it increases permeability in some species and decreases it with others. Information of this sort can be expected ultimately to give us important clues as to the nature of the plasma membrane.

Reference may also be made to another group of facts. In some species, of which man is the best example, permeability of the erythrocyte to glycerol is enormously reduced by the minute traces of copper which occur in some samples of distilled water. This effect is not produced—at least to anything like the same extent—by any other metal so far investigated except mercury. It is also absent with most of the other penetrating substances that have been studied and it is only found in certain species of animals. It is therefore highly specific both from a biological and from a chemical standpoint.

A somewhat similar effect is produced by changing the reaction of the external medium. In general—though the parallelism is not perfect—increased acidity greatly retards the entrance of glycerol in the same species in which it is retarded by copper and it fails to do so in those that are unaffected by copper. This behavior suggests that perhaps proteins may play a more important part in the surface of some erythrocytes than of others, but this is as yet mere speculation. There is, however, evidence obtained by Schmitt, Bear and Ponder[9] by studies of

TABLE II. *Time of hemolysis in seconds at 20°C in 0.3 M glycerol buffered with 0.02 M phthalate and phosphate buffers.*

pH	BEEF	PIG	DOG	MAN	RAT
4.0	1800	910	880	925	720
4.4	2210	1095	995	1095	900
4.8	2524	1170	1120	1140	949
5.2	2720	1190	1150	1065	900
5.6	2772	1240	1175	940	750
6.0	2929	1300	1190	250	320
6.2	2895	1320	1335	50	155
6.6	3114	1320	1408	47	21
7.0	3270	1410	1398	43	20
7.4	3450	1490	1350	35	18
7.8	3630	1545	1350	34	18

birefringence that both proteins and lipoids are present in the surface of the erythrocyte of the rabbit.

Many other facts of this sort might be mentioned. As yet they are more or less isolated, but I believe that with the assistance of the brilliant researches of Dr. Langmuir on artificial films of known composition they may some day be made to fit together like the pieces of a jigsaw puzzle and give us a clearer understanding of the nature of the remarkable membranes that so profoundly modify diffusion processes in living systems.

An entirely different sort of modification of diffusion processes in the erythrocyte results from the presence in this cell of hemoglobin, a substance of high molecular weight which exists in part as an ionized potassium, or in some species, sodium salt. Hemoglobin ions are quite unable to diffuse across the normal cell membrane. However, though unable themselves to do so, they nevertheless modify the distribution of diffusible ions in the manner predicted from thermodynamic considerations by Gibbs and by Donnan. The erythrocyte is, in fact, the classical example of a cell in which the presence of an indiffusible ion may prevent the attainment of an equality of distribution of ions which are themselves freely diffusible. It is also an excellent example of a cell in which through the operation of the same principle a diffusion of ions against a concentration gradient may be made to occur. This principle introduces interesting possibilities for the explanation of apparently anomalous diffusion processes, particularly in cells in which by metabolic activity a more or less steady state of diffusion with respect to the $H^{\cdot}$ ion is main-

tained. These possibilities, however, can only be hinted at here.

As an example of a different sort of complication of diffusion processes in the erythrocyte, I may mention a case involving an osmotic flow of water.[10] In general, the erythrocyte is a very good osmometer, swelling and shrinking in solutions of, for example, NaCl or sucrose of different osmotic pressures in accordance with the usual osmotic laws. Suppose, however, that a suspension of erythrocytes be brought into osmotic equilibrium with a sugar solution of some desired concentration and that after the cells have assumed a constant volume the osmotic pressure of the external solution be suddenly increased by the addition to it of a small amount of a concentrated solution of NaCl. It might be expected that under these conditions the cell would shrink; what actually happens, however, it that it swells, as is shown in Fig. 2, which is a

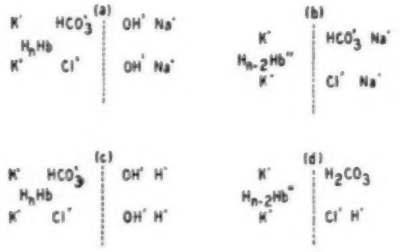


FIG. 3. Probable mechanism of the shrinkage of erythrocytes in an alkaline solution ((*a*) and (*b*)) and in an originally neutral nonelectrolyte solution ((*c*) and (*d*)).

photographic record of the behavior of beef erythrocytes first equilibrated with an isosmotic sugar solution to which NaCl is added at the point indicated by the arrow. Though under these conditions there is a very brief initial shrinkage, shown in the figure, this is promptly succeeded, if the concentration of the NaCl be not too great, by an even greater swelling.

The most probable explanation of this apparently anomalous osmotic behavior of the erythrocyte—which involves a knowledge of some of the properties of the hemoglobin—may be summarized in Fig. 3. Within the erythrocyte some of this substance exists as an uncombined weak acid, represented by the formula H_nHb; like other weak acids, it is able to form ionized salts with potassium. There are also present in the cell other salts of the type KCl and $KHCO_3$. Now let the erythrocyte be exposed to a solution containing an excess of OH' ions (Fig. 3, (*a*) and (*b*)). In a cell permeable to anions under these conditions OH' from outside must be exchanged for Cl' and HCO_3' from inside. But such an exchange leads to the production of dissociated KOH which combines with H_nHb with the disappearance of the OH' ion to form water. The cell therefore loses Cl' and HCO_3' ions without anything to replace them osmotically, and its internal osmotic pressure is correspondingly diminished; shrinkage must follow as a matter of course.

It is not necessary, however, that the external solution should be alkaline to produce this effect. An originally neutral solution of a nonelectrolyte can do the same thing (Fig. 3, (*c*) and (*d*)). Such a solution contains the two ions $H^{\cdot}$ and OH'; of these, OH' enters the cell in exchange for Cl' and HCO_3, and the external solution can readily be shown with a glass electrode to become more acid within a few seconds. The time required for this *p*H change corresponds approximately to that of the shrinkage of the cell, which can be followed by the method employed in Fig. 2. Both *p*H and volume changes are readily and rapidly reversed by the addition of an electrolyte to the sugar solution; in fact such a reversal is the most plausible explanation of the anomalous swelling of the erythrocyte just described.

If time permitted, other examples could be given of the manner in which simple diffusion processes in the erythrocyte may be modified by the presence at the surface of the cell of a membrane, and in its interior of a dissolved substance, hemoglobin, both possessing highly characteristic properties. In ordinary living cells conditions are of course enormously more complex than they are in the erythrocyte. Instead of only a single membrane at the outer surface of the cell we have many such membranes—presumably of different kinds—about the nucleus, about vacuoles, and about all sorts of other structures. Instead of only a single substance like hemoglobin in the interior of the cell to complicate the distribution of other substances there are a very large number. Instead of the practical absence of metabolic activity there are present an infinite variety of complex chemical processes

yielding energy in various ways and maintaining diffusion gradients that could not otherwise exist. Obviously the study of the erythrocyte can be expected to carry us only a very short step in the direction of an understanding of the bewildering complexity of the diffusion processes in ordinary living cells. Many more such steps will have to be taken before we can even partially answer the question raised at the beginning of this paper. But with the properties of Dr. Langmuir's films of known composition and structure as the best possible starting point, and with the erythrocyte as a very convenient first stepping stone, progress towards our distant goal may prove to be more rapid than at one time seemed possible.

Bibliography

1. H. Fricke, J. Gen. Physiol. **9**, 137–152 (1925).
2. J. F. McClendon, J. Biol. Chem. **69**, 733–754 (1926).
3. E. Gorter and F. Grendel, J. Exp. Med. **41**, 439–443 (1925).
4. R. Collander, Soc. Scient. Fennica Comment. Biol. **II**, **6**, 1–48 (1926).
5. S. Mudd and E. B. H. Mudd, J. Gen. Physiol. **14**, 733–751 (1931).
6. M. H. Jacobs and D. R. Stewart, J. Cell. Comp. Physiol. **7**, 351–365 (1936).
7. H. Ulrich, Pflüger's Archiv. **234**, 42–50 (1934).
8. A. K. Parpart, Biol. Bull. **71**, 387–388 (1936).
9. F. O. Schmitt, R. S. Bear and E. Ponder, J. Cell. Comp. Physiol. **9**, 89–92 (1936).
10. M. H. Jacobs, A. K. Parpart and S. A. Corson, J. Cell. Comp. Physiol. **9**, 177–190 (1937).

Uncommon sense is now needed in many situations. We should be stimulated by authority but not paralyzed—admire but not worship. Every advance in science has been at the expense of someone's reputation as an authority. The best plan is to keep the mind free—consciously keep it from crystallizing around formulas of any kind except as working hypotheses which it may be necessary to scrap tomorrow.

—Nolan Don Carpentier Lewis

Electrical Signs of Biological Activity*

By HERBERT S. GASSER

The Rockefeller Institute for Medical Research
New York, N. Y.

THIS symposium, which has been designed to bring together the physical and biological sciences, comes at an appropriate time as it follows within a few weeks the celebration in Bologna of the two hundredth anniversary of Galvani, the physiologist who not only made the first observations of the manifestation of tissue activity that has been assigned to me for presentation here today, but who was also responsible, although indirectly, for the development in nineteenth century physics which had to take place before any measurements of that activity could be made. Electrophysiology has been closely related to physics from the very beginning. At the beginning also it became the target for searching criticism, for the physiologist of Bologna found a worthy antagonist in the physicist of Pavia. It matters not now that the denial of animal electricity was unjustified. The manner of the denial was magnificent. As Volta became harder and harder pressed by the outcome of more ingeniously devised physiological experiments, he in the end was forced to postulate current production by a chain of three components of the second class. Had he gone one step further and located his chain within the tissues and identified them as part of the tissue morphology, he would have been brought to the point of admitting the existence of animal electricity on the basis of his own line of reasoning. Indeed, he would have arrived at the position that we hold today and anticipated the work of a century later.

As it was, we were obliged to wait for the heat laws of Gibbs and Helmholtz, the ionic dissociation theory of Arrhenius, the calculations of the potential of concentration cells by Nernst, the description of the effect of a semi-permeable membrane between two solutions by Ostwald, and the demonstration of a potential at phase boundaries by Haber.

Volta's criticism, potentially constructive, failed to exert the influence that was within the range of possibility, because of his failure to appreciate the situation as it appeared through the eyes of a physiologist; and his eloquent scorn succeeded only in arousing antagonism. The first step in the solution of biological problems is a clear understanding of the problems as they are presented by tissues. Without it, discoveries in the domain of physics can never be expected to attain in biology the degree of usefulness of which they are capable.

The methods of electrophysiology are in theory simple. With proper precautions leads are made from the tissue to a galvanometer or an oscillograph, and the potential is recorded. In general the potentials are of two interrelated types: relatively static potentials indicating states, and transient potentials accompanying action. The problem is then to ascertain where in the tissues the potential actually arises, and to figure out what condition could exist there to develop it. Hypotheses are set up and further experiments undertaken to test the validity of the assumptions made. The procedure is indirect, but represents a methodology not unknown to physicists. In the end the recorded potential serves only to show that something has happened and when it happened. Interpretation depends upon the conditions otherwise incident to the experiment.

* Symposium on Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania, November 4–6, 1937.

The electrical manifestations of irritable tissues have much in common, whether the consideration be of cells of the plant *Nitella* studied

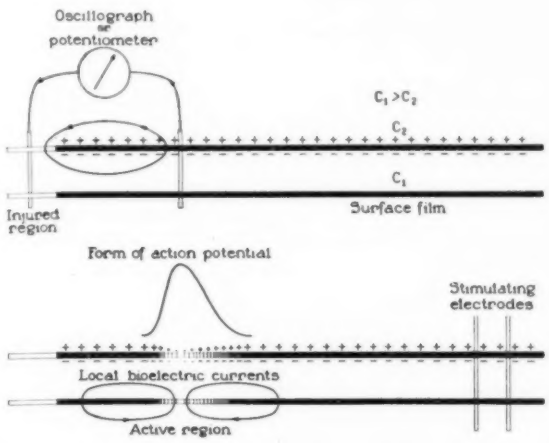


FIG. 1. Diagram of a nerve fiber showing the potential difference between the outside and the inside of the surface film. The probable origin of the potential in the difference in concentration of potassium ions on the two sides of the film is indicated, and the method of revealing the potential by injury of a portion of the surface is shown. How the action potential would be produced by a transient change in the surface is pictured in the lower part of the diagram. The action which started from the stimulating electrodes is presented as traveling toward the injured end.

by Doctor Osterhout and his colleagues,[22] or of the nerves of mammals. In the present discussion nerve fibers will be drawn upon principally for illustrations, as the phenomena which take place in nerve fibers have been more extensively studied than those occurring in other tissues, and because nerve fibers occupy a position of special interest, in that they provide at least qualitative samples of the reactions that are fundamental to all the intricate working of the nervous system.

Biologically speaking, nerve fibers are simple in constitution, as they are only branches of cells. As physical systems they present greater complexity. In a moment it will appear that the reactions which take place in nerve do so with great precision and in a reproducible manner. Reactions of this kind are hardly conceivable except as they have a basis in structure. The inside of nerve fibers is a soft gel (de Rényi[9]), and in the large fibers of some invertebrates it has been shown to be so fluid that it can flow out from the cut end (J. Z. Young[25]). Consequently, the structure must be sought in the surface film.

Many lines of evidence converge to support the assumption that the surface of the fiber is the position most responsible for its activity. The barrier that exists there to the passage of ions, the resistance interposed to the passage of an electric current, and the ready polarizability—all set the surface apart from the interior. Any interference that modifies these properties at the same time alters the manifestations of activity, and the change does not need to be great for the capacity of the nerve to transmit messages to be destroyed altogether (Bishop[4]).

By virtue of the arrangement of its components a nerve fiber has the form of a tubular condenser. When an e.m.f. is applied to two points on its surface, the potential spreads in the extrapolar stretches in the general manner that would be expected for a conductor with a continuous linear capacity. The size decrements with distance and the steepness of rise of potential diminishes.[7] The exact form of the spread is unknown, but it probably does not fit the prediction of the ordinary cable equation, as there is evidence that the capacity is a function of the time.[20] (At this point I must make clear, in order to avoid confusion, that the analogy between a nerve and a cable applies only to spread of polarization. Transmission of messages in nerve involves processes entirely different from anything that takes place in a cable.)

The condenser-like properties of the nerve surface, as well as the potential difference across the surface, are extremely labile. They disappear rapidly when the nerve is deprived of oxygen,[13]

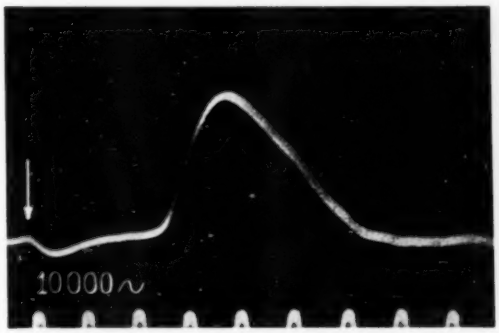


FIG. 2. Record of a spike from a single mammalian nerve fiber. The arrow marks the moment of stimulation. The spike appears after an interval made up of the sum of the latent period at the stimulating cathode and the conduction time between the point stimulated and the locus of the lead.

and are maintained in their normal steady state only through a continuous expenditure of oxidative energy.

One of the consequences of this metabolism is the maintenance of an electrolyte composition on the inside of the fiber different from that on the outside (Fig. 1). Herein lies the most probable source of the potential. The principal ion concerned is potassium. According to the estimations of Fenn and his colleagues, the concentration of K^+ is about 65 times as large inside the fiber as outside. This difference would give 105 mv in a concentration cell and is ample to account for the potential drop across the surface of the fiber which is 30 mv or a little larger. According to this idea, the surface of the fiber would act in the manner of a potassium electrode as a detector of concentration differences, reporting the result in potential. If the concentration of K^+ ions is increased in the external medium by the addition of potassium salts, the potential promptly falls, as the hypothesis demands. The mechanism by which the detection takes place is not certain, but we can look to the sieve membrane[10, 21] or liquid chain models[3] for analogies—three-component systems, of the sort Volta postulated. Whether or not part of the potential may arise from oriented molecules in the surface, in the manner of the experiments on surface films of Schulman and Rideal,[24] has up to the present time been given scant consideration, as there has been little need to go beyond the properties of a concentration chain to find a satisfactory theoretical interpretation of the events that take place in nerve. With the necessary assumptions, a theory set up on this basis would be workable and the subject deserves further investigation, especially since Blinks[6] has found instances among the large plant cells *Halicystis* and *Valonia* in which application of the concentration-cell hypothesis encounters considerable difficulty.

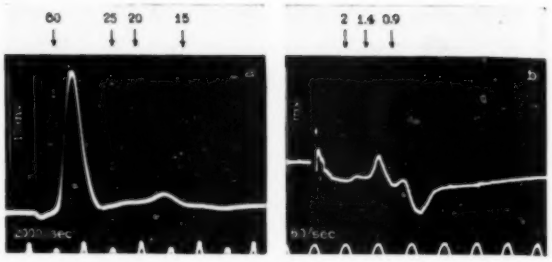


FIG. 4. Action potential in the saphenous nerve of the cat as recorded after 4 cm of conduction from the stimulating cathode. (*a*), the first disturbance in the line marks the moment of stimulation, and the interval between this point and the spike is occasioned by the conduction time of the fastest fibers. (*b*) is recorded at greater amplification and slower sweep speed. The record starts with the potentials in the faster group which carries the oscillograph spot far off the record. The potential in this group ends with a negative after-potential followed by a positive after-potential. In the course of the latter the slow spikes appear. The latter in turn leave a large positive after-potential behind them. At the top of the figure the position of impulses at various velocities is indicated. Record made at 37°C with the nerve in 5 percent CO_2 and 95 percent O_2.

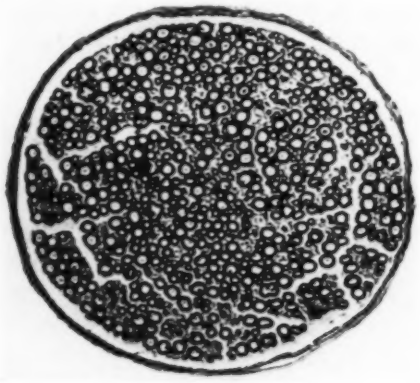


FIG. 3. Cross section of a bundle of fibers in the saphenous nerve of the cat. Osmic acid stain. The nerve axons are the clear spaces inside of the black rings, which are the myelin sheaths. The large fibers are responsible for the first high elevation in Fig. 4(*a*), and the small fibers for the later low elevations. With this stain the nonmedullated fibers which give the delayed elevation in Fig. 4(*b*) are not visible. ×162.

A favorite mode of regarding the potential is from the standpoint of diffusion. Potassium is the only cation that has any considerable mobility in the surface, and the surface is impermeable to anions. Under the condition that one of the ions is immobile, a diffusion potential has the same magnitude as a concentration potential, and thus the situation at the surface of the fiber would be accounted for. The high concentration of potassium inside the fiber as compared with that in the tissue spaces implies the operation at some period in the life of the nerve of a mechanism of the nature of a pump. Also, if at any time in the course of nervous activity potassium under the pressure of the concentration gradient should leak past the surface barrier, it would have to be restored by a similar mechanism.

In order to reveal the potential difference across the surface of the fiber, electrodes are placed on intact and injured portions of the nerve; the electrode on the injured region serves as a lead to the inside of the surface (Fig. 1). When a fiber becomes active, this difference undergoes a transient decrease; and if the electrodes are connected to an oscillograph, there is recorded a spike-like variation which resembles

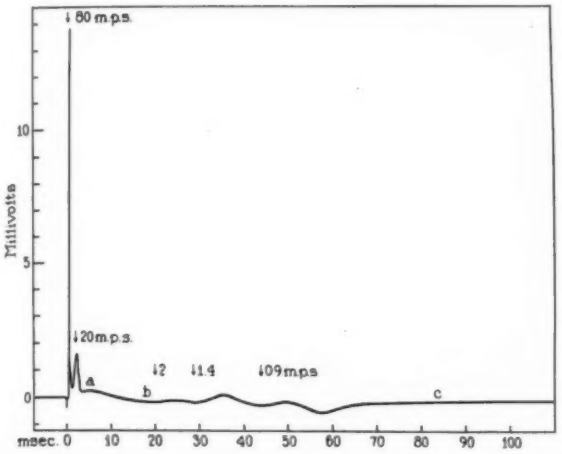


FIG. 5. The potentials shown in Fig. 4(*a*) and (*b*), drawn in relation to a single amplification and time scale. (*a*) Negative after-potential in the fast fibers. (*b*) Positive after-potential in the fast fibers. (*c*) Positive after-potential in the slow fibers.

somewhat a skew probability curve. In mammalian nerves this variation lasts about 0.4 msec., of which time one-third is taken up by the phase of development (Fig. 2). Nerve messages are carried by a succession of these spikes.

What is the change in state of the fiber that permits the development of a spike? When we ask this question we ask the principal question of nerve physiology; and at the outset I may say that we cannot answer it. However, the process is not mysterious. It is possible to think of ways in which it could happen. The difficulty lies in the fact that we can think of too many ways, without being able to establish the validity of any one of them. The events happen rapidly and within a space of small dimensions possibly measurable in angstroms. Electrical measurements settle the time and help in other ways, but exact information about some of the other aspects of the change at the moment seems as remote as measurement of the potential must have seemed to Galvani after his disheartening experience with the gold-leaf electroscope.

A number of facts guide our thinking about the matter. Over a wide range of velocities the duration of the spike is constant; and in a given fiber the constancy is maintained throughout a wide variety of experimental conditions. Some process in the fiber has a time course intrinsically determined and not easily disturbed. A change in duration is readily produced only by alterations of pressure and temperature, a high pressure[14] acting like a low temperature. The temperature coefficient, Q_{10}, for the change in duration of the spike is about 3 in the normal working range, but it mounts rapidly at lower temperatures, especially just before extinction of excitability supervenes. While the behavior resembles most closely that of the viscosity of a lipoid,[11] the involvement of chemical reactions is not excluded.

Another illustration of the extent to which the spike is determined by the intrinsic properties of the nerve is found in the law of all-or-nothing. If the spike appears at all, it appears at the maximal size which the momentary condition of the nerve will permit.

The disturbance once started spreads over the nerve fiber at constant velocity and without change in form or size. It is dependent entirely on the local condition and independent of the

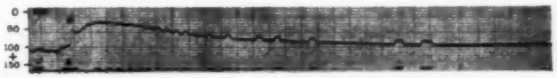


FIG. 6. Change of polarizability of *Nitella* during activity. A lead between the intact surface of the cell and a killed end shows the side to be strongly positive to the end. At 1 the surface is polarized with an outwardly directed current (cathodal polarization). At 2 a stronger current is applied, and in the course of the polarization an action starts (at *s*), resulting in a marked decrease in the potential difference. With currents of the same strength as at 2 the polarizability is tested at various times during the action. It will be noted that at the crest of the action the polarizability is lost, but that it gradually returns as the resting state is restored (L. R. Blinks[6]).

nature of the stimulus which produced it. If it is made to traverse a depressed region, it again becomes full-sized when it emerges into normal nerve. In other words, the energy for the event is supplied locally. The system has the appear-

ance of having potential energy stored ready for release at the arrival of the impulse.

With regard to the mechanism of propagation, one theory dominates all others. It is based on the fact that the most effective artificial stimulus

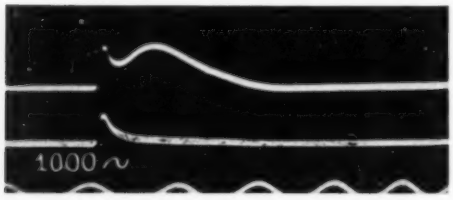


FIG. 7. Polarization and action potentials in frog nerve at 15°C. The lower curve shows the polarization potential at the cathode when a just subthreshold induction shock is applied. The upper curve is produced by a shock 1.5 times as large and shows an action potential rising from the polarization potential. The stimulating cathode and the active lead are at the same electrode.

for nerve is the electric current. Extensive investigation of the law connecting the duration of a current with the strength necessary for stimulation has given much information about the excitation process. Only a simple point need be cited for the present argument, however. Excitation takes place where the current passes the surface from inside to outside. Adjacent to an active region the current in the fiber flows in this direction, and this fact is made the basis of the theory (Fig. 1). The nerve is supposed to excite itself by the local current between the active and adjacent inactive regions.

The velocity of propagation depends upon the structure of the fiber; the greater the diameter, the faster is the conduction. According to the local biolectric circuit theory, the current would increase as the square of the diameter, while the surface acted upon would be as the first power. Thus the current density per unit surface would vary as the diameter; and the result observed is not entirely unexpected. Because of the size velocity relationship, the action potential of a mixed nerve has a configuration reflecting its fiber content (Fig. 3). Impulses starting out together at one point on the nerve become temporally dispersed as conduction proceeds, and the action potential appears with a number of elevations. Figs. 4 and 5 show the picture obtained from the saphenous nerve of the cat, which is a typical sensory nerve.

In part *a* velocities between 80 and 15 meters per second are represented. A group of potentials in large fibers with a maximal velocity of 80+ m.p.s. makes up the first high elevation. Following this come a number of smaller elevations made up of velocities between 30 and 15 m.p.s., with a peak component at 20 m.p.s. The second group represents as large a number of fibers as the first group, but the combined potential is small because of the small size of the fibers involved. The slowest group in the nerve conducts at velocities between 2 and 1 m.p.s., with the major component at 1.4 m.p.s. Unmyelinated fibers[15] with constants differing from those holding for the spike in Fig. 2 and the combined potentials in Fig. 4 (*a*) are responsible for this elevation.

Nerves going to muscles carry impulses at a velocity faster than any found in sensory nerves going to the skin. When leads are taken from a mixed nerve, velocities as high as 120 m.p.s. are found. From the size velocity relationship it follows that the wave-length also varies as the diameter. At the upper end of the series the disturbance extends over about 5 cm of fiber, while at the lower end of the series it is shorter than 1 cm.

In addition to the potential changes, two other electrical measurements have been made of an active region. The observations of Lullies[20] on nerve and of Blinks[6] on *Nitella* supply evidence that during activity both the resistance and the polarizability of the surface decrease (Fig. 6).

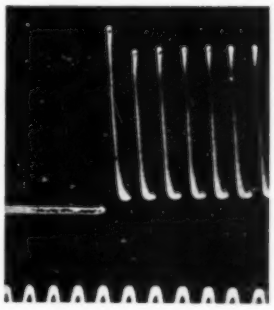


FIG. 8. Tetanus at 1075 per second. Phrenic nerve of the cat at 37.5°C in 5 percent CO_2 and 95 percent O_2. Time, 1000 cycles. The fibers are still partially refractory at this interval and the responses after the first one are only 91 percent of normal size. A progressive decrease in size occurs at this frequency in isolated nerves.

Thus activity is associated not only with a loss of potential, but with disintegration of the condenser. Of the two, the latter is especially significant.

As a final point it should be mentioned that the energy cost of nervous transmission is low. When a nerve is experimentally subjected to an intensity of activity far beyond the range of normal, the metabolic increase which the activity entails amounts to only about 10 percent of the resting value, and this in experiments made under conditions in which it would be expected that the increase would be higher rather than lower than that under perfectly physiological conditions. A nerve's expenditure of energy is principally for maintenance. From the heat measurements of A. V. Hill and his colleagues[16] we can form an idea of the time at which the energy is expended. Nearly all (95 to 98 percent) of the heat is produced after the period of transmission is over, and there is even some question of whether any heat is liberated during the spike. The methods of analysis require a half-second tetanus, and as on electrical evidence the recovery processes start in the first millisecond, there is good reason to believe that a part at least of the heat produced in the initial period is recovery heat. In any case, the heat measurements show clearly that the energy expenditure is mainly in connection with processes operating to restore the state which obtained before the activity started.

From the foregoing set of observations we may now try to put together a picture of the sequence

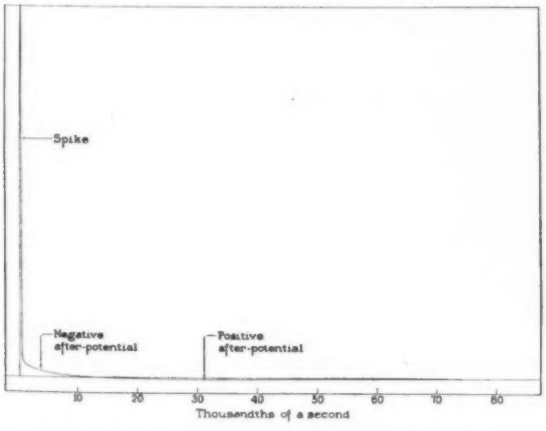


FIG. 9. Course of the potential cycle in a mammalian nerve fiber in a single response.

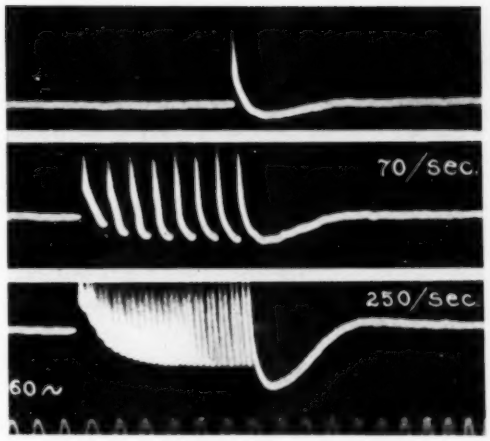


FIG. 10. Changes in the after-potentials in the phrenic nerve produced by short tetani. The records start with the negative after-potential (see Fig. 9); the spikes extend far above the records. Nerve at 37°C in 5 percent CO_2 and 90 percent O_2.

of events in hypothetical form. As a starting point an applied potential or the local bioelectric current may be selected. Nernst originally pointed out that the first effect of a current would be to bring about an accumulation of ions at barriers, but how this accumulation would lead to excitation was not made clear. In the short utilization period of the exciting current the accumulation of ions could not be great, and it seems worth while to consider the distribution of potential within the circuit. With a source of potential of about 30 mv and with the greater part of the resistance of the circuit at the surface of the fiber, opportunity is present for large potential drops (1 mv per 100A is 1000 v per cm). In fields of this magnitude one would expect reorientation of dipoles, either as a direct effect of the field or secondary to an alteration of the dipole moments resulting from chemical reactions such as oxidation or reduction and saponification or esterification—or from release of adsorbed ions. For one reason or another the arrangement of the molecules in the surface film would be upset and a mechanism put before us whereby the known changes characteristic of the active surface could be brought about: the decreased resistance, capacity and potential. A current would now flow through the disturbed segment from the adjacent region, *just as it would if the segment were placed under mechanical strain*. As a result the adjacent region would be

excited; and through a succession of excitations, conduction would be effected.

Excitation by a polarizing current presupposes that a potential above a certain critical value has been applied and that a critical quantity of current has flowed. When this has happened, the surface film has been brought into a metastable state ready for the change. Continuation of the polarizing current is unnecessary and the spike may appear with a latency after the current is withdrawn. When the spike appears it rises sharply from the polarization potential which preceded it (Fig. 7).

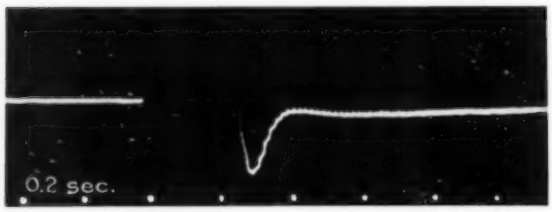


FIG. 11. After-potentials in a phrenic nerve after a longer tetanus. The tetanus starts at the break in the line and the first visible part of the potential is produced by the negative after-potential on the last spike. The first sharp positive potential corresponds to that seen in Fig. 10. It is succeeded by a second positive potential of much longer duration (Gasser and Grundfest).

At this stage we come to the most obscure part of the cycle. What brings about restoration? The inflowing current in the local circuit would help, but it could operate only after the restorative process, for other reasons, would be well under way. Restoration, as a matter of fact, can still occur if the polarizing potential which effects the excitation is allowed to act throughout the cycle. Displacement and restitution must be intimately coupled. They both have the same temperature coefficient.[11] The sequence has a precise tempo—witness the constancy of the spike. And restitution to a degree permitting another response is prompt. Fig. 8 shows nearly full-sized spikes following one another at 1075 per second.

As considered thus far, there is little about the events described that suggests an evolution of heat. Any energy liberated at the breakdown of the surface would be counterbalanced so soon by an equivalent absorption on reformation that the sequence would show no change by any known method of measurement. The local bioelectric current, if it arises as believed from a concentration cell, should cause the cell to cool. But the cooling would be balanced by the Joule heat equivalent of the current, as in the experiments of Bernstein and Tschermak[2] on the electric ray, *Torpedo*. The electric organ of this animal cooled if the discharge was expended outside of the organ; and it failed to do so if the discharge was expended within it. Thus initial heat, if indeed there be any initial heat, would suggest the intervention of a chemical reaction,[17] either to start the surface change or to bring about its restoration.

More reasons can be seen for a delayed metabolism. The active state is conducive to a decrease in the potassium gradient between the inside and outside of the fiber. The part of the surface yielding current could do so only by an increase of potassium concentration outside and a decrease inside. Also the lowered permeability in the active region would permit diffusion outward, although here diffusion would be opposed by the incoming current. The metabolism continuously going on to maintain the concentration difference, therefore, would have to be increased. Actually the mechanism must be very efficient, as the increase in oxygen utilization is small, and the amount (if any) of potassium ordinarily leaking out is not detectable chemically. That potassium can leak out, however, is evident from Cowan's[8] observations on crab nerves.

All the delayed heat production cannot be concerned with the replacement of potassium, as the late part of the potential picture is not what one would expect from this hypothesis. At the

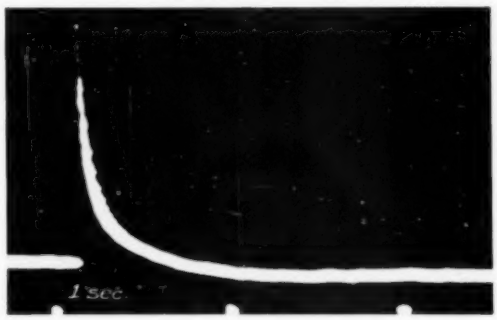


FIG. 12. Prolongation of the after-potentials by veratrine. Single response in a phrenic nerve. (Gasser and Grundfest.[12])

subsidence of the spike the potential cycle as a whole has only begun. The 0.4 msec. spike of mammalian fibers is succeeded by a long low potential lasting from 80 to 100 msec. The last spike of a tetanus may be succeeded by a potential lasting a minute, and a tetanus of an isolated frog nerve may be succeeded by a potential lasting from 10 to 15 minutes.

The after-potentials run a characteristic course. In a mammalian nerve in normal physiological condition the spike is succeeded by a negative after-potential lasting about 15 msec., and this is followed by a positive potential lasting until about 70 msec. have elapsed (Fig. 9). The positive potential is relatively fixed in its time course, but not as fixed as the spike with which it appears to be in continuation. Following a tetanus it is augmented in size but not prolonged (Fig. 10), and it is then succeeded by a second positive potential which increases in size and duration in proportion to the duration of the tetanus (Fig. 11). The negative after-potential is variable. At times, as in veratrine poisoning, it is greatly prolonged; and when this occurs it is succeeded by a delayed positive potential which would correspond to the second positive potential after a tetanus (Fig. 12). At other times, as when the nerve is in tetany,[18] the whole after-potential system oscillates (Fig. 13).

To follow the after-potentials through all their complex behavior would serve no useful purpose at the present time. Suffice it to say that their importance in the physiology of the nervous system lies in the fact that they determine the level of the nerve's excitability. Their interest in the mechanics of nerve lies in their reflection of the nerve's metabolism.

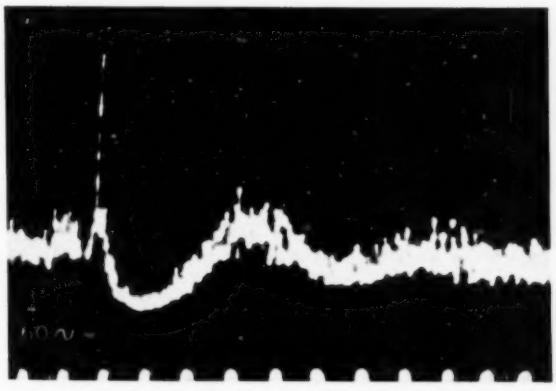


FIG. 13. Oscillating after-potential in the phrenic nerve of the cat. Nerve mounted in air at 37°C. The spike is not visible and the record starts with the after-potentials. Spontaneous firing of the fibers occurs. During the supernormal phases at the crests the firing increases, while during the subnormal phases in the troughs it decreases. Oscillation is also visible at normal reaction, but it is too small to show satisfactorily in a reproduction.

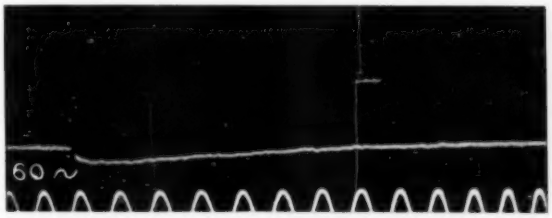


FIG. 14. Spike and positive after-potential in a group of fibers in the hypogastric nerve of the cat. Velocity of conduction 12 m.p.s. and slower. Note the relative absence of negative after-potential and the large size of the positive potential with respect to the height of the spike.

Measurements of the heat production of nerve are always made during and after a tetanus. The durations of the delayed heat given by A. V. Hill[17] correspond with our own observations of the duration of the after-potentials under comparable conditions, and in addition there is evidence of a correspondence between the rate of evolution of heat and the configuration of the potential. The heat curve is described by the sum of two exponentials, one decrementing rapidly, the other slowly. The rapidly decrementing part would correspond to the early portion of the potential where the negative after-potential is visible, leaving the slowly decrementing part to cover the later period of positivity. Other evidence connecting the after-potentials with metabolism is found in the augmentation of metabolism occurring when the after-potentials are increased and the spikes are unchanged,[23] and in the differential depression of the after-potentials with respect to the spikes in asphyxia.[1, 23]

In a more intimate analysis of the after-potentials their existence can best be accounted for on the basis of the state of the surface. More than one factor must be involved. The first positive after-potential after the spike may be an overshooting in the restoration of the surface. It shows very clearly in the potentials of small mammalian fibers in which there is little negative

after-potential (Fig. 14). An analogy is found in the thickness of the oxide film formed on the surface of iron passivated in strong HNO_3. Professor Lillie[19] has called attention to the fact that after a wave associated with reduction has passed over the surface, the oxide coat is thicker at the end than it was at the beginning. In this regard, as in many other ways, Professor Lillie's iron wire model presents remarkably close analogies to the events taking place in nerve fibers.

Additional events in the after-potential are not difficult to visualize. Alteration of metabolism in the nerve would mean a change in the molecular species competing for position in the surface, and depending upon the changing composition of the surface film a varying report, in the form of a potential, would be rendered about the existing concentration difference. In view of the complex behavior of the after-potentials, the electrical evidence for the simultaneous existence of more than one process and the rhythmicity, the potential changes seem to be more readily explainable by alteration of the surface film than by a shift of ions as between its two borders. Alteration in the content and arrangement of the molecules in the surface could bring about a change of intrinsic origin in the potential drop across the film, subject to the reservation that the magnitude of the observed change would be related to the composition of the adjacent phases. As an extrinsic effect the change in mobility of potassium ions in the surface would cause an impairment or improvement of the film as a detector of a concentration difference resulting in an appropriate shift of the recorded potential. Very little movement of ions would need to take place.

The story that I have patched together is much like a wall in which the cement substance is too great for the number of bricks. It may not stand up. But if it turns out that I have gone afield in the interpretations, in compensation I hope that I have succeeded in demonstrating that nerve activity is nothing more than the operation of a mechanism—complex to be sure, but one working in an orderly manner according to laws the definition of which need not be considered as altogether beyond the powers of fine physical measurements.

References

1. W. R. Amberson, A. Parpart and G. Sanders, Am. J. Physiol. **97**, 154 (1931).
2. J. Bernstein and A. Tschermak, Pflüger's Arch. **112**, 439 (1906).
3. R. Beutner, *Die Entstehung elektrischer Ströme in lebenden Geweben* (Ferdinand Enke, Stuttgart, 1920).
4. G. H. Bishop, J. Cell. Comp. Physiol. **1**, 177 (1932).
5. L. R. Blinks, J. Gen. Physiol. **16**, 147 (1932); **18**, 409 (1935).
6. L. R. Blinks, J. Gen. Physiol. **20**, 229 (1936).
7. J. Y. Bogue and H. Rosenberg, J. Physiol. **82**, 353 (1934).
8. S. L. Cowan, Proc. Roy. Soc. **B115**, 216 (1934).
9. G. S. de Rényi, J. Comp. Neurol. **47**, 405 (1929).
10. U. Ebbecke, Zeits. f. Biol. **91**, 247 (1931).
11. H. S. Gasser, Am. J. Physiol. **97**, 254 (1931).
12. H. S. Gasser and H. Grundfest, Am. J. Physiol. **117**, 113 (1936).
13. R. W. Gerard, Am. J. Physiol. **92**, 498 (1930).
14. H. Grundfest, *Symposia on Quantitative Biology*, Vol. 4 (Cold Spring Harbor, 1936) p. 179.
15. P. Heinbecker, J. O'Leary and G. H. Bishop, Am. J. Physiol. **104**, 23 (1933).
16. A. V. Hill, Proc. Roy. Soc. **B111**, 106 (1932); **113**, 345 (1933).
17. A. V. Hill, *Chemical Wave Transmission in Nerve* (Cambridge University Press, Cambridge, England, 1932).
18. J. E. Lehmann, Am. J. Physiol. **118**, 600 (1937).
19. R. S. Lillie, Biol. Reviews **11**, 181 (1936).
20. H. Lullies, Pflüger's Arch. **225**, 87 (1930).
21. L. Michaelis, Kolloid Zeits. **62**, 2 (1933).
22. W. J. V. Osterhout, Physiol. Rev. **16**, 216 (1936).
23. F. O. Schmitt and H. S. Gasser, Am. J. Physiol. **104**, 320 (1933).
24. J. H. Schulman and E. K. Rideal, Proc. Roy. Soc. **A130**, 259, 270 (1931).
25. J. Z. Young, J. Physiol. **83**, 27P (1934).

Potential Energies
of
Biologically Important Oxidation-Reduction Systems*

By WILLIAM MANSFIELD CLARK

DeLamar Professor of Physiological Chemistry
Johns Hopkins University, School of Medicine, Baltimore, Maryland

TIME is not one of the dimensions ordinarily used in thermodynamics. Therefore it is convenient to regard this science as one that deals with potential energies, in the most general sense. It has no *direct* concern with the mechanisms or the time relations in the display of energy changes.

On the other hand the living cell exhibits chemical changes that are more or less continuous with respect to time. This is particularly true of those several distinct types of chemical change that are integrated in providing the energy of muscular contraction, and the energy of secretory and other processes. Accordingly there is a tendency to regard chemical kinetics as especially pertinent to this part of biochemistry and to set aside those parts of thermodynamics that treat true equilibrium states; leaving only the final energy changes to be considered.

A noteworthy exception obtains whenever electrolytes come into consideration. In these systems the interaction of components is so rapid that equilibria can be assumed to be established. Changes with respect to time are considered to be shifts of true equilibrium states and in the last analysis are treated by methods rooted in thermodynamics.

We may grant the limitations of chemical thermodynamics on the one hand and of chemical kinetics on the other and still take advantage of what each offers. For example, thermodynamics of itself can contribute little of a positive nature to our knowledge of chemical mechanism. Yet consider its negating value in the following instance. A wrongly interpreted experiment purported to show that metallic palladium can extract hydrogen from hydroquinone. This alleged fact continues to be used in support of the hydrogen transport theory of oxidation-reduction in organic systems. An examination of the thermodynamics of the hydroquinone experiment shows that so large a quantity of energy would have to be expended that the probability of the process occurring in the direction specified falls in the same category as the probability that a kettle of water will boil when placed on ice.

This is but one striking example of the fact that neglect of energy relations has permitted the postulation of highly improbable and practically impossible mechanisms. In less conspicuous cases the excuse sometimes offered is that, amid the complexity of the living cell, several reactions may be coupled in such a way that the energy made available by some is used to make possible the others.

This is all very well as a glittering generality but I for one prefer to wait for the concrete evidence of how the energies of different processes are coupled. We now have a few cases some of which I shall mention briefly later.

Hitherto we have been dependent upon heats

* Presented at the Symposium on Biophysics, Philadelphia, Pennsylvania, November 4, 5, and 6, 1937.

of combustion of organic compounds for the thermodynamic data applicable to biological problems. Heats of combustion serve very well in animal calorimetry but in the first place none of the machines of the body is a heat

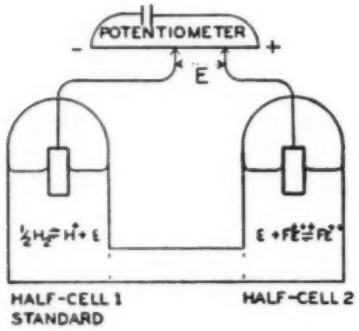


FIG. 1. An electric cell.

engine and in the second place it is not the heat change, ΔH, but rather the free energy change, ΔF, that determines chemical change for which a free path is open; when the conditions are constant pressure and temperature.

By use of entropy measurements a few data on free energy changes pertinent to biochemistry are accumulating. In certain instances, the ordinary electric cell can be used. We shall confine attention to this device.

Consider the standard oxidation-reduction system

$$2H^+ + 2 \text{ electrons} \rightleftharpoons H_2,$$

where H^+ is the oxidant and H_2 the reductant. Consider any other oxidation-reduction system, for example

$$Fe^{+++} + e \rightleftharpoons Fe^{++}.$$

If these two systems are mixed they will not interact at a rate sufficient to establish equilibrium rapidly. By providing a catalyst for the hydrogen system the potential energy of the interaction between the two systems is released and an equilibrium state obtains.

Now isolate the two systems as in the electric cell shown in Fig. 1. The liquid junction shall be such as to prevent direct interaction, it shall offer passive resistance to the *independent* migration of electrons there, it shall provide for the completion of the electric circuit by the migration of ions and, for present purposes, we shall assume that it is so designed as to reduce the diffusion potential to a small, indeterminate, and now negligible value. The metallic part of the circuit acts as a filter that permits only electrons to flow. Thus the energy of action is directed into an "electrical" channel and is measurable with great precision.

When the electric cell is in potentiometric balance there is provided the condition for the retention of the chemical energy as potential energy. Then, if chemical potentials are defined as the increase of free energy per chemical equivalent, it can be shown that the product of the faraday, F, and the electric potential difference, E, is a direct measure of a certain set of differences among the chemical potentials of the participating components of the two oxidation-reduction systems.* We then derive the

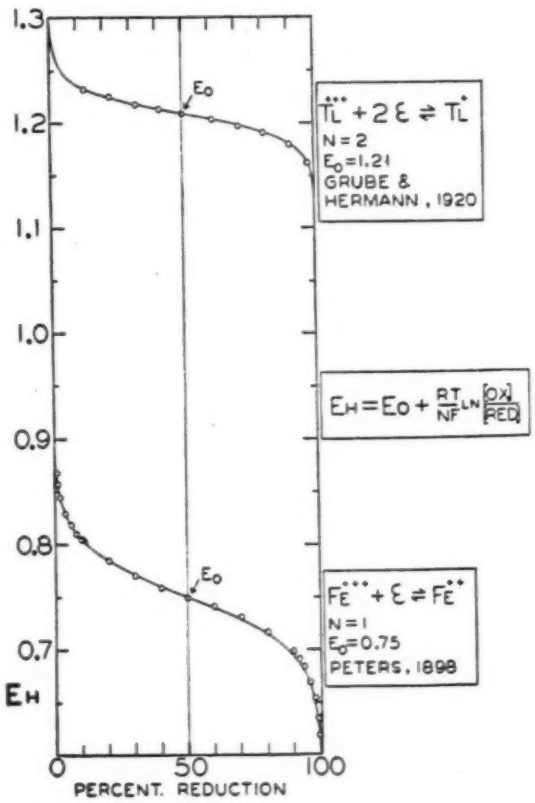


FIG. 2. Relation of observed potential E_h to percent reduction in the cases of two inorganic systems.

* See Clark, *Harvey Lectures* (1933–34).

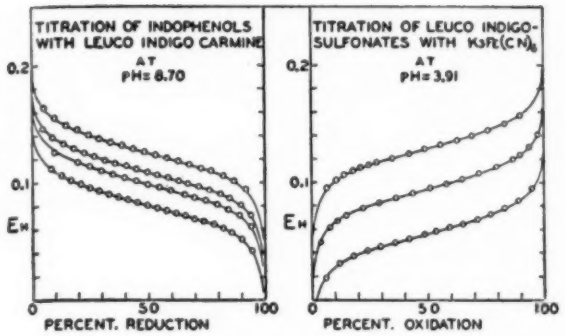


FIG. 3. Examples of conformity of experimental data to the type curve ($N=2$).

following relation involving concentrations [ ] of components.

$$FE = FE_0 + RT \ln \frac{(H_2 \text{ pressure})^{\frac{1}{2}}[Fe^{+++}]}{[H^+][Fe^{++}]}. \quad (1)$$

For the sake of simplicity we now neglect activity coefficients. These usually can be collected in a separate term and made the subject of a special study.

$FE = FE_0$ is the potential energy in volt-faradays that would be released were hydrogen at one atmosphere to be converted to one mole of hydrogen ions at unit concentration with the simultaneous conversion of a mole of ferric ions to a mole of ferrous ions.

Remove the configuration of the electric cell and the established value of the free energy change in volt-faradays FE still remains a thermodynamic characteristic of this chemical process by whatever mechanism it takes place elsewhere. Therefore do not assume that, because we shall use particular terms relating to the method of study, these terms necessarily imply anything regarding electrical phenomena in the living cell. When this symposium was being arranged Dr. Bronk threatened to put this paper in a section on the electrical properties of the living cell. I told him that if he did so I should have to explain how I got into bad company.

The process of comparison, in principle, consists in substituting one oxidation-reduction system after another in the second half-cell represented in Fig. 1.

Transposing F in Eq. (1) and assuming the components of the standard system are H_2 at 1 atmosphere and H^+ at 1 molar we have

$$E_h = E_0 + (RT/F) \ln [Fe^{+++}]/[Fe^{++}]$$

or at 30°C.

$$E_h = E_0 + 0.0601 \log [Fe^{+++}]/[Fe^{++}].$$

In general and with qualifications to be mentioned presently

$$E_h = E_0 + (RT/nF) \ln [\text{Oxidant}]/[\text{Reductant}].$$

Just as in general mechanics we find potentials more generally useful than potential energies, so, in these cases, we find the electrical potential more useful. It represents the intensity factor of the potential energy. E_h (the subletter of which refers to the system of standardization) will now be referred to for brevity as "the potential."

Figure 2 shows data for the iron and thallium systems. The system that is higher in the scale is potentially able to oxidize the system lower in the scale. Appreciable action in the opposite direction is impossible. Thus a placement of all oxidation-reduction systems provides a means of predicting the direction of interaction of any two if a mechanistic path be open. By considering the relative quantities of components, the possible extent of action in the required direction can be accurately calculated.

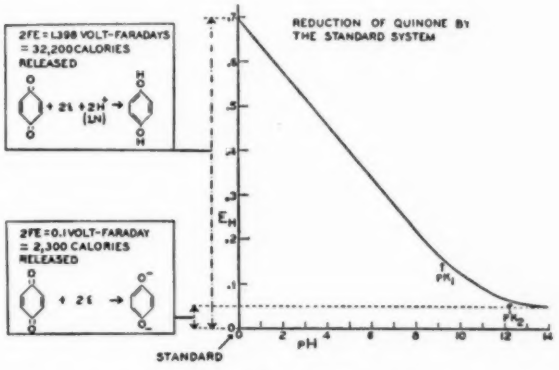

2FE = 1.398 VOLT-FARADAYS = 32,200 CALORIES RELEASED
2FE = 0.1 VOLT-FARADAY = 2,300 CALORIES RELEASED
REDUCTION OF QUINONE BY THE STANDARD SYSTEM
EH
pH
STANDARD

FIG. 4. Effect of pH when (total oxidant)/(total reductant) = 1.

The same device functions with certain organic systems. Since this field has been reviewed frequently I may be permitted to give only two illustrative sets of experimental data from my own laboratory, exhibited in Fig. 3.

Here it will be noted that the *p*H values of the solutions are carefully specified.

One of the most interesting aspects of the study of organic systems is the clarity with which the effects of shifting acid base equilibria are exhibited. For the sake of simplicity I shall now omit details and shall make but one comment.

With the aid of Fig. 4 consider the comparatively simple case of the quinone-hydroquinine system held at 50 percent reduction. When quinone, O=⬡=O, is reduced to hydroquinone in the electric cell, we can assume for purposes of analysis that electrons come via the metal from the hydrogen system to form $^{-}$O⬡O^{-}. To complete the formation of hydroquinone, hydrogen ions must be taken from other components of the solution to form HO⬡OH. Now the availability of the hydrogen ions in the solution is measured by the hydrogen electrode potential and is conveniently expressed in terms of *p*H, which is a linear function of the hydrogen electrode potential. As the availability of the hydrogen ion is lowered (increase of *p*H) more energy is used in their acquirement and less is available in the external circuit of the cell. This is reflected in the linear decrease of potential with increase of *p*H exhibited in Fig. 4. When the *p*H is so high that hydrions would be stripped from hydroquinone, the potential becomes invariant with change of *p*H.

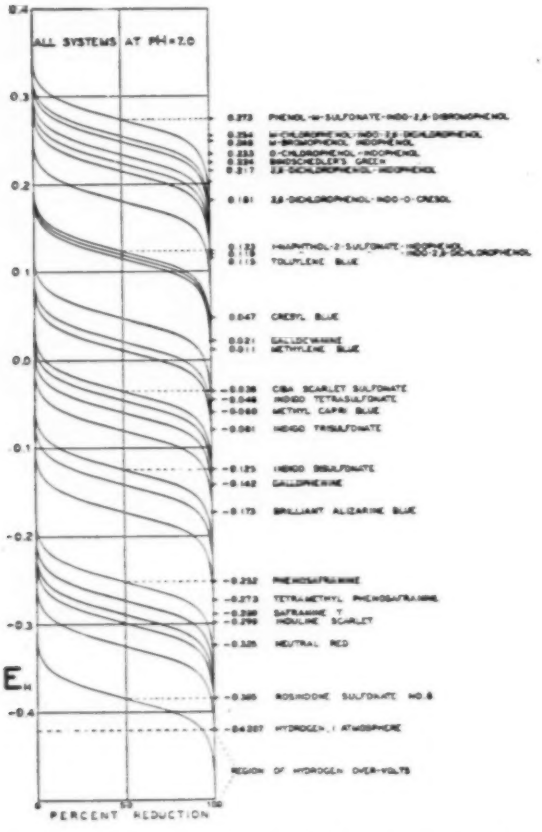


FIG. 5. Oxidation-reduction indicators at *p*H 7.0.

In the complete equations these effects appear as the free energy of hydrogen ion dilution and the free energy of acid ionization. Obviously these acid base phenomena are integrated with the oxidation-reduction process; inseparably so, except insofar as they are revealed by imposing proper control. In this sense we have an intimate coupling of two sorts of processes that have been kept distinct throughout the history of chemistry. To illustrate the coupling I could show, if I had time, the complete reversion of the oxidation of one system by another simply by changing the acidity of the solution. As we proceed we shall see other perfectly definite couplings of the energy changes of other types of chemical action.

To avoid the complexity of acid base changes let us now assume that we are operating at *p*H 7.0 near the hydrogen ion activity usually favorable to life.

Gillespie[16] demonstrated that when a bacterial culture is placed in one half-cell of our standard device the voltage of the cell progressively declines as if intense reducing systems were forming in the culture. Directly after Gillespie's preliminary results Clark[9] made very extensive examinations of this phenomenon and concluded that its interpretation had best await the results of systematic studies. First we developed a series of oxidation-reduction indicators the potentials of which are shown in Fig. 5.

Their conduct in suspensions of cells and, as used by Cohen and others,[11] their conduct within the interior of a living cell, such as amoeba, roughly confirm the electrode measurements. (See Fig. 6.) In the first place everything points to the conclusion that the living cell in aerobiosis is extremely far from being in equilibrium with atmospheric oxygen and that the

catalysis of oxygen utilization probably is such as to avoid the direct activation of the oxygen molecule. Since this subject has not yet lent itself well to the type of study under consideration we may pass to the simpler anaerobic conditions of life which are uncomplicated by the participation of oxygen. There we find high intensity of reduction; so much so that certain organisms attain the theoretical limit for aqueous systems. The behavior of electrodes remind one of the conduct which would be expected in systems intermediate between those which are so electromotively active as to stabilize the potential well and those which have no definite effect whatever. Do cells contain such systems? Let us see what has happened as the subject has developed.

As already indicated, certain types of oxidation-reduction system behave beautifully in the electric cell and permit the measurement of free energy changes with very high precision. Several of these systems have been found in living cells. The list includes several pigments such as the pyocyanin formed by a bacillus, several hydroquinones such as the phthiocol of the tubercle bacillus and our hormone epinephrine. The list includes several hemochromogens and other special agents to be mentioned presently.

Other systems are sluggish in their behavior at the electrode and for convenience are spoken of as only partially electromotively active. Ascorbic acid (vitamin C) is an example. It behaved sufficiently well for preliminary measurement. Finally my associate, Dr. Ball,[1] obtained excellent data by providing a mediator, that is, a system that enters the chemical equilibrium and is itself fully active electromotively.

Other systems by themselves have no definite action on an electrode. Such a system is a mixture of the salts of fumaric and succinic acids. Certain living organs contain a catalyst that displays its activity by the fact that in its presence the succinate-fumarate system reacts reversibly with certain other systems, for example that of methylene blue. Now that the characteristic potential of the methylene blue system is known, its use, together with the equilibrium constant, for the interaction with succinate-fumarate permits the calculation of the characteristic potential of the succinate-fumarate system. Or, one can use the methylene blue in small quantity as a mediator and measure directly the potential of the equilibriated mixture. Table I shows the results of these two types of measurement. Included in this table are the data of Borsook and Schott[8] for ΔF and ΔH obtained by thermal methods. The agreement is good.

The principles have been applied by Barron and Hastings,[5] Green *et al.*,[17] Wurmser and Filitti,[29] Laki,[20] and others, to several systems of biochemical importance. A few of the data are assembled in Fig. 6. Evidently biological catalysts are providing the means of applying the device of the electric cell in somewhat the same way that the catalyst platinum black made possible the use of the hydrogen system.

If anyone is confused by the fact that I have placed along a scale of *electrical* potential the positions of systems that are not electromotively active, the confusion will be relieved by imagining any other unit for the intensity factor of free energy that is equivalent to the volt.

It is particularly pleasing to me to note the calculated position for the system marked with a dot. Eighteen years ago I established that

TABLE I. *Oxidation of succinate to fumarate. Succinate ion$^=$(1M)→fumarate ion$^=$(1M)+2H$^+$(1M)+2ϵ; $-\Delta F = -20{,}140$ calories $= -84{,}270$ volt coulombs. Heat change (difference in heats of combustion). Succinate ion$^=$(1M)→fumarate ion$^=$(1M); $-\Delta H = -29{,}800$ calories. Data reduced to 25°C basis by Borsook and Schott.*[8]

INVESTIGATORS	METHOD	$-\Delta F$	$-\Delta H$
		calories	*calories*
Quastel and Whetham (1924)	Equilibration with methylene blue*	—20,180	
Thunberg (1925–1928)	Equilibration with methylene blue*	—20,100	
Lehmann (1929–1930)	Potentiometric with mediators	−20,180	−29,850
Borsook and Schott (1931)	Potentiometric with mediators	−20,140	
Borsook and Schott (1931)	Thermal	−20,460	−29,800

* Data for methylene blue by Clark, Cohen and Gibbs (1925).

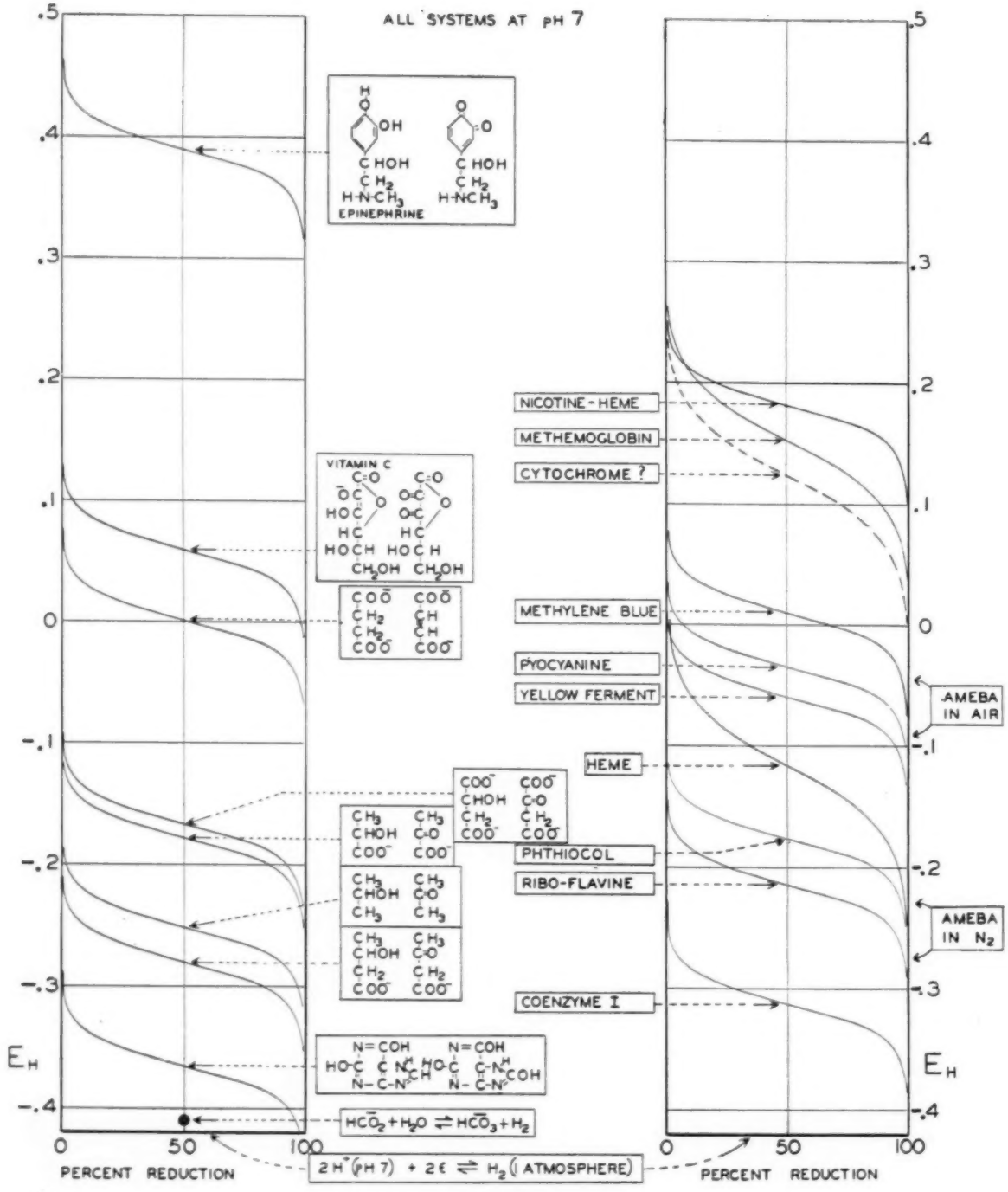


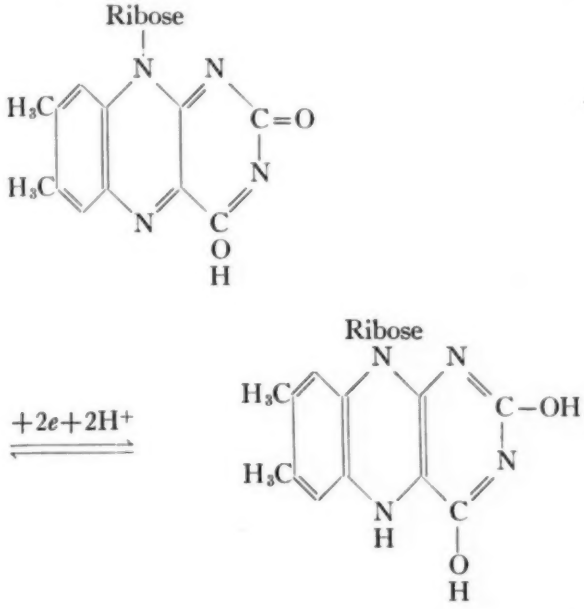
FIG. 6.

bacteria of the colon-aerogenes group can bring the potential of the medium to the region of the hydrogen electrode, without entire dependence on the system $H^+ : H_2$. Previously it was known that they liberate hydrogen from formate. Woods[28] has now shown that they catalyze and make reversible the reaction

$$H\overline{CO}_2 + H_2O \rightleftharpoons H\overline{CO}_3 + H_2.$$

Everything now fits together.

After Warburg and Christian had isolated the so-called "yellow ferment" they found (see Warburg, Christian and Griese[27]) that it contains a group that is subject to reversible oxidation-reduction. It is electromotively active. With respect to the present subject only and without disparagement of the functions of other parts, we may call this the functional group. The structure of this part of the yellow ferment, which can be separated as ribo-flavin, is shown below as a reversible oxidation-reduction system.



The ribo-flavin system above has the potential curve indicated in Fig. 6 (Michaelis *et al.*[23]). Ribo-flavin functions as a vitamin. Its phosphate ester and a specific protein form the yellow ferment, the potential curve of which is distinctly positive to those of the flavines (Kuhn and Boulanger[19]).

Two coenzymes (I of Euler and II of Warburg) have recently been isolated and their components identified. Again, what we may call, for *present* purposes, the functional groups are subject to oxidation-reduction. To my knowledge no accurate value of the characteristic potential has been determined. Nevertheless Euler *et al.*[14] report the establishment of an equilibrium between coenzyme I and the lactate system and, although this selection of the lactate system is unfavorable to the purpose, I have used his data for the calculation of the approximate placement of the potential curve (see Fig. 6). An indication of its validity is that the slope of the change of potential with change of pH is roughly that expected of the process

$$Ox^{+}+2e+H^{+}\rightleftharpoons \text{Reductant}.$$

See Table II.

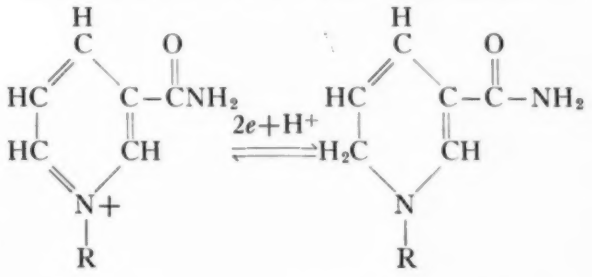
TABLE II.

$$\frac{[\text{Oxidized Coenzyme}]\,[\text{Lactate}]}{[\text{Reduced Coenzyme}]\,[\text{Pyruvate}]}=K.\ K \text{ determined by Euler}$$

pH	E_0' (LACTATE/PYRUVATE)	0.029 log K	E_0' COENZYME	$-\Delta E/\Delta p$H
7.4	−0.175	0.150	−0.325	0.023
8.1	— .216	.125	— .341	—028
8.7	— .250	.111	— .361	.033
				Ave. 0.028

Theoretical $-\Delta E/\Delta p\text{H}=0.029$ at 20° for the process Oxidant $+2e+\text{H}^{+}=$ reductant.

This process is to be expected, on grounds of organic structure, since the coenzymes, which also contain pentose, phosphate, and adenine, have the nicotinic acid amide group which is subject to the following reversible reduction.



In passing, it is interesting to note that vitamin B_1 contains a quaternary nitrogen atom in a position comparable to its position in coenzymes I and II making this compound also subject to reduction, perhaps reversibly. What appears to be the pyrophosphate ester of B_1 (Lohmann and Schuster[21]) functions as a cocarboxylase.

If two oxidation-reduction systems are so constituted that they will not act directly upon one another even in the presence of their respective activating enzymes but when activated will act upon a third system, we may have the following situation described with the aid of

Fig. 7. If the potential curves are placed as shown, their positions predict the directions in which interactions are possible. The placement eliminates consideration of each reaction in the direction opposite to that which is possible. Reverting now to purely kinetic considerations it can be shown that whatever may be the individual rates of action, the mediator system will maintain itself so as to supply continuously oxidant to react with the reductant of the more negative system and reductant to react with the oxidant of the more positive system. Except for initial adjustments and subsequent slow shifts the mediator is maintained in accordance with the classical definition of a catalyst and at the equilibrium point is found at the percent reduction predicted on purely thermodynamic grounds. If the potential of the mediator be outside the intermediate range of the other system, either its oxidant or its reductant may be brought to low concentration and consequently may be less effective. *All other factors being equal* the efficiencies of a series of mediator systems should then vary with the characteristic potentials roughly in the manner illustrated. See also Borsook.[7] It should be carefully noted that we are dealing here only with the most elementary aspects of the subject and are not entering the deeper question of the relation of oxidation-reduction potentials to absolute rates discussed, for example, by Gershinowitz.[15] Green and Dewan[17] are assembling a series of cases in which this concept of the rôle of a mediator has proved valuable in experiments *in vitro*. Euler *et al.*[14] have used coenzyme I in establishing equilibrium between the following systems:

System 1
Reduced and oxidized coenzyme I
Reduced and oxidized coenzyme I
lactate-pyruvate

System 2
lactate-pyruvate
glycerose phosphate-glycerol phosphate
glycerose phosphate-glycerol phosphate

There is a strong suggestion that the active, reversible systems found in living cells play the rôle of catalyzing mediators in accelerating the action of one system upon another. If so no new nomenclature or theory is necessary so far as these are concerned. Their functions are evident and to a certain degree limited specificity of a sort is made evident.

Without attempting the same definite analysis in the case of oxygenation we have to admit that something of a similar nature is revealed by the previous observations of Barron and Hoffman[6] and the recent experiments of Stotz and Hastings.[25] They found that the efficiency of dyes in promoting oxidation of certain substrates by oxygen is a function of their characteristic potentials.

Thus thermodynamics provides certain critical data on the components of biological catalysts without touching the mechanism by which passive resistance to interaction is overcome. The path for action being open, the relative potentials define what reaction can use the path —thus ordering and simplifying the subsequent kinetic treatment.

We may also note a matter of no great importance except to our modes of thinking as represented in current nomenclature. In the course of the historical evolution of the subject it became customary to chose an oxidant, such as methylene blue, to test the activation of a substrate, such as lactate, which is a reductant. When action was found it was natural to say that this *reductant* is activated. What was overlooked was the simultaneous participation of the corresponding oxidant, e.g., pyruvic acid,

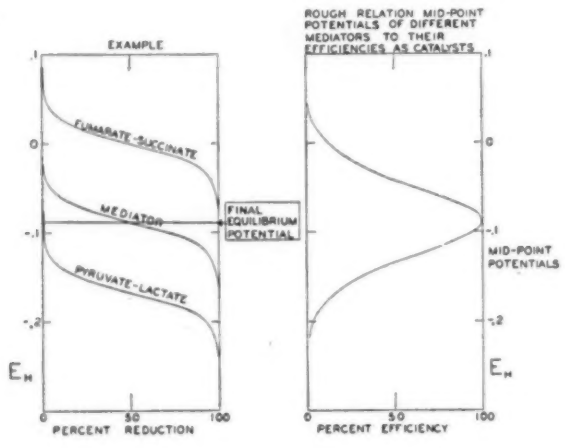


FIG. 7.

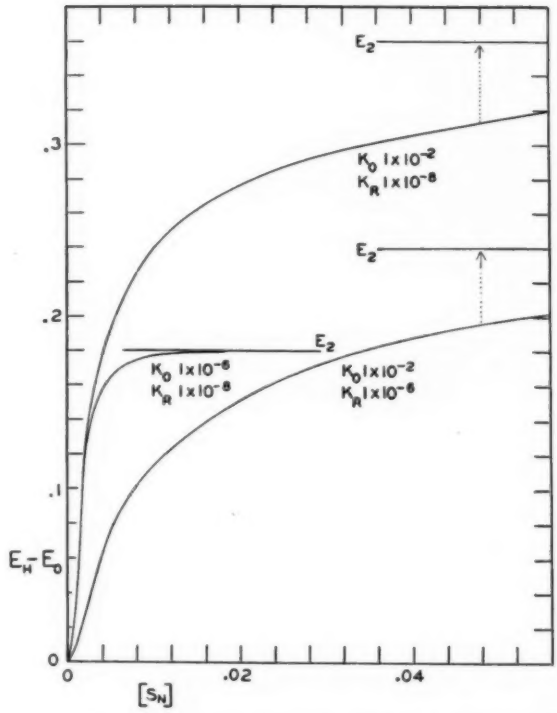


FIG. 8. Relation of electrode potential E_h (relative to the potential E_0 in the absence of associating base) to the concentration of total base $[S_n]$. System at constant percent reduction. Effect of varying relative and absolute magnitudes of the dissociation constants of the complexes; K_0 for the oxidized metalloporphyrin-base complex, K_r for the reduced metalloporphyrin-base complex.

exhibited in the approach to equilibrium from either starting material. Couple the first narrow point of view with the narrow mechanistic theory of hydrogen transport and one finds the basis of the present confusing nomenclature. We could get along much better by discarding the current nomenclature of the subject and reverting to plain descriptions of established facts.

The fact that several of the systems that function as catalysts are themselves electromotively active might suggest that electromotive activity has something to do with catalytic activity. Electromotive activity of two systems separately does not necessarily mean that they will react upon one another rapidly as Shaffer[24] has emphasized. Did time permit it would be interesting to dwell upon Shaffer's concept that rapidity of action is determined in part by the higher probability of bimolecular collision in which there should be equivalence of the electrons given up and accepted.

We should then discuss at length the cases uncovered by Michaelis[22] and by Elema.[13] In certain instances the thermodynamic data of equilibria at progressive stages of reduction take a form that demands the postulation of a special mechanism. The answer given is that there is a splitting of the stages of reduction providing an intermediate. Thus, certain systems can provide either one or two electrons.

Whatever may be the ultimate bearing of the facts upon theories of mechanism it cannot be denied that it was an essentially thermodynamic study of equilibrium states that revealed the necessity of postulating new species.

To keep the emphasis upon certain central and simple principles we need not consider now the complications that arise when one component of a system disappears as it is formed; by entering another reaction. This sort of situation is, of course, that which will have to be considered in metabolic studies. But in passing I might say that we have used potential measurements successfully in following the rates of disappearance of one unstable component of a very active system and have deduced some of the consequences of such a situation. See Ball and Chen.[2]

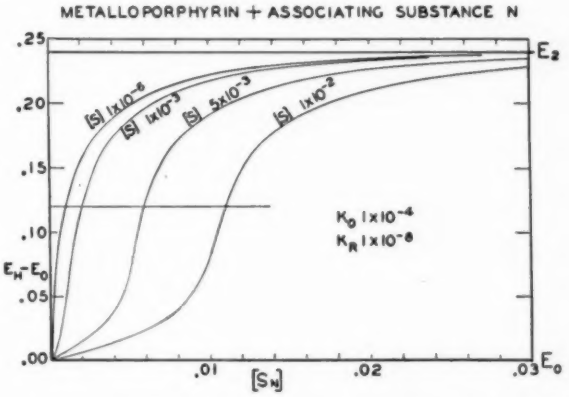


FIG. 9. Comparable to Fig. 8 but showing the effect of varying the concentration of total metalloporphyrin $[S]$.

A noteworthy feature of the "yellow ferment" is the dependence of its catalytic activity upon a reversible association between the part which, alone or in combination, can function as an oxidation-reduction system, and a specific protein.

According to one concept the coenzymes also associate with specific proteins. It has been

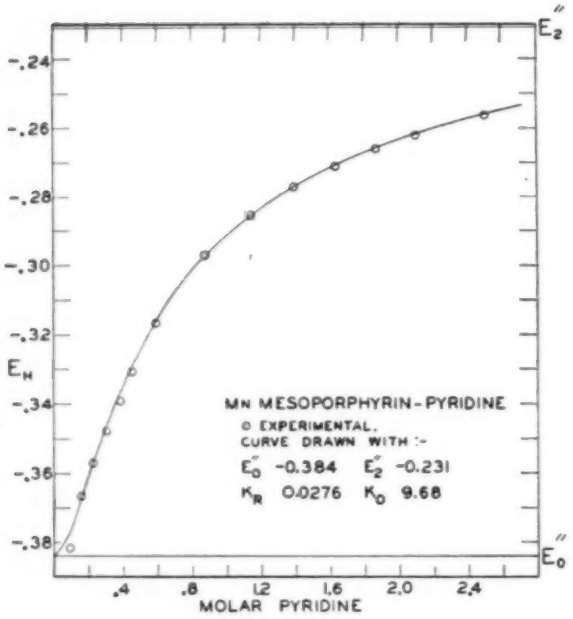


FIG. 10. Coordination of metalloporphyrin with nitrogenous base.

suggested that several oxidation-reduction catalysts are associations between specific proteins and compounds such as characterize the yellow ferment and the known coenzymes.

Thus the catalytic function of proteins enters the field of oxidation-reduction as it has in so many instances in the field of the biological hydrolytic agents, and gives a possibly general significance to the following.

One of the most striking properties of the metalloporphyrins is their reversible association with various substances, particularly organic bases, and certain proteins. Thus hemoglobin consists of ferrous protoporphyrin and the protein globin. Cytochrome and some other natural catalysts are also protein hemochromogens. Through the researches of Conant and his co-workers and more recently of Barron[3] heme systems are known to be electromotively active and to involve the following processes: (1) reversible oxidation and reduction of the metallic component; (2) reversible association between metalloporphyrin and organic base (or protein); (3) reversible ionization of acidic and basic groups; (4) reversible aggregation in certain instances.

The electrode potentials reflect the integration of these diverse types of chemical action and, before we can trace the effect of each, most elaborate controls must be instituted.

Barron's[3] recent paper will show the effect of different associating bases upon the potential of systems in which heme is the functional group.

We have succeeded in deriving equations for such cases and we have verified them experimentally by selecting metalloporphyrins that in some respects are more favorable to the study of the association.

A positive displacement of potential on addition of nitrogenous base is due to the fact that the association between base and reduced metalloporphyrin is greater than the association between base and oxidized metalloporphyrin. Fig. 8 shows the theoretical effect of changing the association constants, all other factors remaining constant. Fig. 9 shows that when one system is employed the potentials are a function of relative concentrations.

Figure 10 shows the effect of added pyridine in a case studied by Taylor.[26] The curve was drawn with the aid of rather elaborate equations that need not now be given. Plotting the same data in Fig. 11, the abscissa of which is the logarithm of the difference between the concentrations of total base and total metalloporphyrin, we obtain a curve that is subject to graphical analysis. E_0 is the potential of the metalloporphyrin system in the absence of associating base. E_2 is the potential when both oxidized and reduced metalloporphyrin are saturated with associating base. The tangent to the

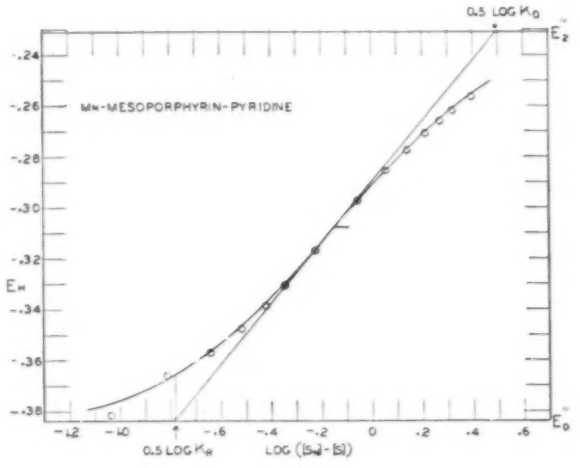


FIG. 11.

curve has its slope determined by the number of molecules of base associating with one of metalloporphyrin—in this case 2. The intersection of the tangent with the line of E_0 gives the value of 0.5 log K_r where K_r is the constant defining the dissociation of the metalloporphyrin base complex in reduced form. The intersection with the line of E_2 likewise allows the calculation of K_0, which refers to the dissociation of the oxidized complex. With constants determined by graphical analysis we can calculate the distribution of species as a function of potentials as is done in Fig. 12 for the case of 50 percent reduction.

Barron and Hastings[4] have shown that the shift of potential resulting from the addition of nicotine changes the heme system from a poor mediator of oxygenation to a good one—in line with the behaviors of dyes of different characteristic potentials.

Future considerations of this phenomenon will be from the points of view of mechanism and energetics. Those who follow the latter will not overlook the very great energy changes associated with the combination of protein and

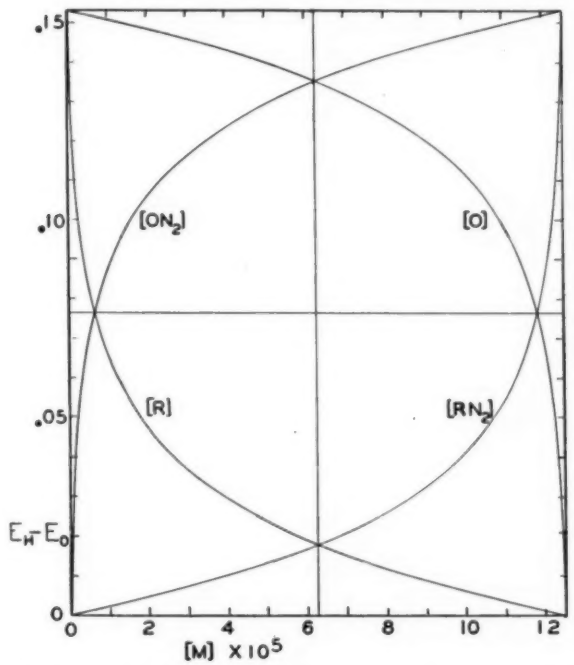


FIG. 12. Distribution of species as a function of potential. Total manganese mesoporphyrin 12.5×10^{-5} molar.

[O]	concentration of	oxidized	metalloporphyrin
[R]	"	reduced	"
$[ON_2]$	"	oxidized	complex with 2 pyridines
$[RN_2]$	"	reduced	" " " "

"prosthetic" group. At long last we now have quantitative data on this that moves the subject a little way out of the field of pure speculation and reveals the integration of this other sort of process with those already treated.

Another suggestion of what the future may hold is the revelation by my associates, Dr. Hellerman and Dr. Cohen, that certain hydrolytic and related processes are controlled by the state of reduction of the catalyst. See Hellerman's review.[18]

The student of muscle metabolism will be quite dissatisfied with what has been considered here because no mention has been made of the rôle of phosphorylation in the oxidation-reduction of metabolites, and because I have not considered certain minutiae in the interaction of the systems mentioned. Time has not permitted the discussion of certain relations and regarding others we have no data.

Lack of data has not restrained speculation within the field of oxidation-reduction for nearly a hundred years. But if there is one thing the new technique has provided it is definite numerical data upon which reason can proceed safely for a short distance. To go beyond would violate the spirit of the new approach and rob the future of its chief attraction.

References

1. E. G. Ball, J. Biol. Chem. **118**, 219 (1937).
2. E. G. Ball and T-T. Chen, J. Biol. Chem. **102**, 691 (1933).
3. E. S. G. Barron, J. Biol. Chem. **121**, 285 (1937).
4. E. S. G. Barron and A. B. Hastings, J. Biol. Chem. **100**, 155 (1933).
5. E. S. G. Barron and A. B. Hastings, J. Biol. Chem. **107**, 567 (1934).
6. E. S. G. Barron, and L. A. Hoffman, J. Gen. Physiol. **13**, 483 (1930).
7. H. Borsook, *Ergebnisse der Enzymforschung IV*, (1935).
8. H. Borsook and H. Schott, J. Biol. Chem. **92**, 535 (1931).
9. W. M. Clark (1919); Abstracts Bact. **4**, 2 (1920).
10. W. M. Clark, *Harvey Lecture* (1933–4).
11. B. Cohen, R. Chambers and P. Reznikoff, J. Gen. Physiol. **11**, 585 (1928).
12. J. G. Dewan and D. E. Green, J. Biochem. **31**, 1074 (1937).
13. B. Elema, Rec. trav. chim. **50**, 807 (1931).
14. H. v. Euler, E. Adler, G. Günther and H. Hellström, Zeits. f. physiol. Chem. **245**, 217 (1937).
15. H. Gershinowitz, J. Chem. Phys. **4**, 363 (1936).

16. L. J. Gillespie, Soil Sci. **9**, 199 (1920).
17. J. G. Green, J. G. Dewan and L. F. Leloir, J. Biochem. **31**, 934 (1937) (and previous articles by Green *et al.*).
18. L. Hellerman, Physiol. Rev. **17**, 454 (1937).
19. R. Kuhn and P. Boulanger, Ber. **69B**, 1557 (1936).
20. K. Laki, Zeits. f. physiol. Chem. **249**, 63 (1937).
21. K. Lohmann and P. Schuster, Naturwiss. **25**, 26 (1937).
22. L. Michaelis, J. Biol. Chem. **92**, 211; **96**, 703 (1931–2).
23. L. Michaelis, M. P. Schubert and C. V. Smythe, J. Biol. Chem. **116**, 587 (1936).
24. P. A. Shaffer, J. Am. Chem. Soc. **55**, 2169 (1933).
25. E. Stotz and A. B. Hastings, J. Biol. Chem. **118**, 479 (1937).
26. J. Taylor, *Dissertation Johns Hopkins University* (1937).
27. O. Warburg, W. Christian and A. Griese, Zeits. f. Biochem. **279**, 143 (1935).
28. D. D. Woods, J. Biochem. **30**, 515 (1936).
29. R. Wurmser and S. Filitti, Compt. rendus soc. biol. **118**, 1027 (1935).

In any attempt to make science more effective in industry and through it more helpful to the public, certain obstacles must be met and overcome. First I would mention the so-called "hard-headed practical business man;" a man without vision, imagination or enthusiasm for new things; a man who scoffs at theory or a college degree; a man whose sole criterion of proper practice is that which he has been accustomed to in the past; a man who spends as little as he can on research in order that his profits day by day may be larger. The withering policies of such men have driven many a flourishing business into obsolescence. If, by accident, a research laboratory has been set up in this man's company, its staff will be among the first to be fired in a depression, thus saving temporarily dollars but losing permanently the capital investment in trained intelligence.—KARL T. COMPTON—"Science in an American Program for Social Progress"—*Scientific Monthly*

Optical Studies of the Molecular Organization of Living Systems*

By FRANCIS O. SCHMITT

Department of Zoology
Washington University, St. Louis, Missouri

WHEN we review the history of biological thought dealing with the physical structure of living cells and tissues we see that for a time following the discovery of the cell, about a century ago, observations were made almost entirely on living cells. Then came the discovery that by fixing and staining the cells with a variety of reagents and dyes a host of new and intriguing structures could be demonstrated. Thus the classical methods of histology originated and in the heyday of this period, from the seventies to the nineties, schools of thought arose which attempted to characterize the fundamental structure of protoplasm according to certain geometric types, as exemplified in the fibrillar, alveolar and granular theories.

With the advent of colloidal chemistry it became clear that much of this cytological work was merely a study of fixation artifacts. In the revolt which set in, led by the biochemists, structure meant little and life became a "dynamic equilibrium in a polyphasic system" (Hopkins). More recently, however, thanks largely to x-ray diffraction work, we appear to have entered upon a new era ushered in by the modern knowledge of the molecular organization of highly polymerized compounds such as the proteins. As a result of these revelations the cytologist has regained a certain measure of confidence in the fundamental nature of his studies. It has been found, for example, that when a structure consistently, and with a variety of techniques, takes on fibrillar shape, this is indeed due to the fact that the molecules themselves are arranged in longitudinal array; the molecules are themselves fibrous.

However, valuable methods of classical morphology may be in *suggesting* the structure of protoplasm, the only escape from the indeterminacy inherent in the method is the study of fresh, unfixed cells and tissues. Fortunately, methods are available which are capable of giving precise information as to the molecular organization of living systems and which avoid serious thanatological consequences because they involve only the exposure of the cells to light, and in particular only to those portions of the spectrum which are not greatly absorbed, namely visible light and x-rays. In the present discussion we shall call attention to the polarized light and x-ray diffraction methods of analysis of tissue fine structure and shall indicate briefly some of the results which have been obtained.

Remarks on the Polarized Light and X-Ray Diffraction Methods

Since the technique involves a rather specialized branch of optics a few preliminary words by way of orientation may be desirable.

If a system is constructed in a manner such that the electric fields of the constituent molecules are asymmetric, the body is anisotropic.

* Presented at the Symposium on Biophysics, Philadelphia, Pennsylvania, November 4, 5 and 6, 1937.

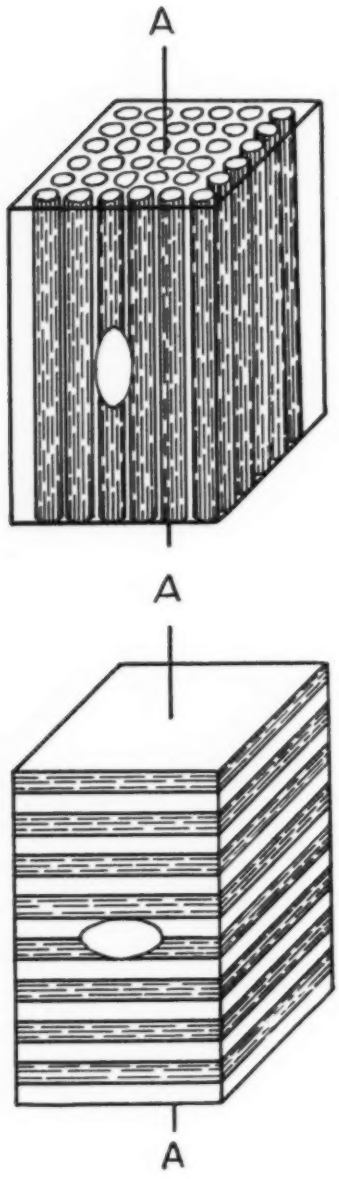


FIG. 1. The Wiener expression for the form birefringence of ideal two component mixed bodies consisting of oriented rodlets or platelets. Upper diagram shows rodlet form birefringence

$$Ne^2 - No^2 = \frac{\delta_1\delta_2(N_1^2 - N_2^2)^2}{(\delta_1+1)N_2^2 + \delta_2 N_1^2}.$$

Lower diagram shows platelet form birefringence

$$Ne^2 - No^2 = -\frac{\delta_1\delta_2(N_1^2 - N_2^2)^2}{\delta_1 N_1^2 + \delta_2 N_2^2}.$$

If a beam of plane polarized light be passed through such a system, and if the vibration of the beam is not parallel or perpendicular to a direction of symmetry, it will be broken up into two mutually perpendicular components which are oriented for fastest or slowest transmission through the system. The system therefore has two refractive indices rather than one and is said to show double refraction or birefringence. The magnitude of the birefringence is equal numerically to the difference between the two descriptive refractive indices.

The object is said to show positive birefringence with respect to some distinctive direction, such as the long axis of a fiber, if the refractive index is a maximum in this direction and a minimum in the direction perpendicular thereto. If the refractive index has a minimum value in the fiber axis and a maximum value in the perpendicular direction, the fiber displays negative birefringence with respect to the fiber axis. If the direction of the optic axis is known, the sign of birefringence should be that referred to this direction.

In practice, to determine the birefringence, the refractive indices are seldom measured. Instead, the phase difference or the retardation of the one ray with respect to the other is measured by a suitable compensator. Knowing the characteristics of the compensator, the sign of the birefringence can also be determined.

One customarily thinks of optical anisotropy as due to a regular orientation of molecules in a crystalline or semi-crystalline state (intrinsic or "Eigen" birefringence). However, if the system contains bodies which are small with respect to the wave-length of light, are asymmetric in shape and are preferentially oriented the system may show birefringence whether the particles themselves are crystalline or isotropic. Since biological systems are almost invariably composed of such mixed bodies, this so-called "form birefringence" is of considerable importance.

From the theory of form birefringence as elaborated by Wiener[1] a number of important facts may be deduced about the ultrastructure of a tissue. The Wiener equations and their applications are shown in Fig. 1. In these equations n_e and n_0 are the refractive indices for the extraordinary and ordinary rays, respectively, n_1 and n_2 the refractive indices of the particles and the surrounding medium, respectively, δ_1 and δ_2 the partial volumes of the particles and

surrounding medium, respectively. It will be noted that the form birefringence of systems composed of rodlets (e.g., particles with long axes paralleling the optic axis of the system) is always positive while that of platelets (e.g., particles with long axes perpendicular to the optic axis) is always negative. The sign of the form birefringence is therefore diagnostic of the configuration of the system.

To determine whether the birefringence of a tissue or cell component is due to the intrinsic or to the form factor or to both one has only to immerse the material consecutively in fluids of different refractive indices, measuring the birefringence after each fluid has had time to penetrate into the system. Plotting the observed birefringence against the refractive index of the medium one can deduce from the curve the relative importance of each factor. If the birefringence is due exclusively to the intrinsic factor it will show no dependence on the refractive index of the immersing medium. If the form factor is significant the curve will be similar to those shown in Fig. 2. Curve 2 is that typical of systems in which the birefringence is due exclusively to the form factor (the sign of the birefringence being positive in the case represented by this curve). It will be obvious from the equations that such pure form birefringence will be abolished when the refractive index of the immersion medium is exactly equal to that of the particles (e.g., $n_1 = n_2$). If both form and intrinsic birefringence are present (curves 1 and 3), the magnitude of the intrinsic birefringence is given by the ordinates of the minimum of the curve. The abscissa of the minimum gives the refractive index of the oriented particles (e.g., $n_1 = n_2$), a fact of some value in determining the chemical nature of the particles. In systems showing both form and intrinsic birefringence the sign of the two types may be the same (curve 1) or opposite (curve 3). In certain cases the theory permits an approximate determination of the relative amount (δ_1) of the oriented submicroscopic particles present. The Wiener theory applies, strictly speaking, only to ideal two-component mixed bodies. Biological systems are seldom so simple, being represented usually by a multiple-component mixed body. The analysis of such systems, however, follows along lines similar to those obtaining for two-component systems.

The chief application of this type of analysis for our present purposes will be its value in determining the relative shapes of the ultramicroscopic bodies. If a structure shows positive form birefringence with respect to its optic axis we may conclude that it is composed of particles with long axes paralleling the optic axis; these are usually thought of as rodlets or fibrillae. If the structure shows negative form birefringence with respect to the optic axis we may assume that it contains particles whose long axes are perpendicular to the optic axis; these are usually thought of as platelets or lamellae.

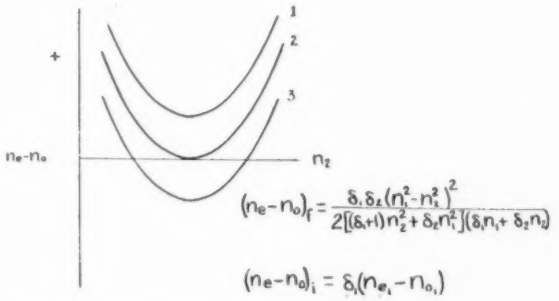


FIG. 2. Form birefringence curves obtained by immersing tissues in media of various refractive indices (n_2). The modified Wiener equation is more convenient practically than the original. Further description in text.

It is perhaps unnecessary to make any explanatory remarks about the x-ray diffraction method, for the technique and theory are essentially similar to those used in ordinary crystal analysis. Since very large spacings occur in animal tissues it is necessary to use long wavelength x-rays (copper or chromium radiation) and the specimen-to-film distance may be as long as 10–20 cm. If a fresh tissue is being studied it must be enclosed in a moist chamber to prevent drying. Fig. 3 shows a moist chamber found useful for work with nerves. A nerve is suspended between the thin glass stirrup, shown on the right of the figure, the nerve being held between the two slender glass rods by the surface tension of a small amount of Ringer solution. This glass stirrup is then inserted into the closely fitting glass chamber shown on the left of the figure. Nerves remain moist in such chambers

indefinitely. The windows of the chamber are of glass 10–20μ in thickness.

The spacings between the planes are usually calculated from the Bragg relation and the types of orientation and molecular configuration in any tissue must be deduced from geometric and chemical considerations of each system individually.

The Molecular Organization of Cellular and Tissue Components

The microscopic and submicroscopic structural elements of animal cells are composed predominantly of protein or of complexes of protein and lipoid; carbohydrates are of importance primarily as the fuel of metabolism. When we survey the various types of structures visible in living cells and tissues or in fixed and stained material we find that most of these structures may be placed in one of two geometric categories: either they show preferential linear arrangement and may be called essentially fibrous, or they are composed of sheets or layers of material and may be called sheaths, membranes or films, depending on their relative thickness. It will be convenient to organize our discussion of tissue fine structure on the basis of these two geometric categories.

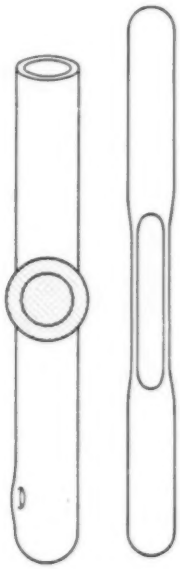


FIG. 3. Moist chamber for prevention of drying of nerve during exposure to x-rays for the purpose of obtaining a diffraction pattern.

1. Linear (Fibrous) Orientation

Fortunately animal fibers lend themselves readily to x-ray diffraction analysis and much work has already been done particularly on hair and the protein textile fibers. From this work it has become clear that the fibers owe their anisodiametric properties to the fact that they are composed of longitudinally oriented string-like protein primary valence chains, the latter being integrated into a fiber by more or less strong lateral linkages through unions of terminal groups of the side chains of the individual amino acids of the primary valence chains. The relative stability of the lateral integration determines the compactness and texture of the fibers, and this in turn depends on the types of amino acids which comprise the protein chains. If the amino acids possess long side chains with polar terminal groups capable of reacting with each other, the compactness, tensile strength, swelling properties, etc., of the fiber will be very different than if the side chains are short and have nonpolar terminal groups. On the basis of such considerations animal tissues and fibers might be classified as follows (see Jordan Lloyd[2]): (1) purely mechanical tissues such as hair, (2) biologically inactive, supporting tissues such as tendon and, (3) active tissues, such as muscle, intracellular fibers, etc. In class 1 the protein chains are compact and well integrated laterally by side chains linkages; there is little room for water and the tissue has practically no metabolism. In class 2, while the metabolism is still very low, the water content is considerably higher and phenomena of swelling are much more pronounced. Class 3 represents the type found in actively metabolizing tissues with high water content and in which the actual configuration of the chains may be altered rapidly and reversibly; the latter is obviously a necessary attribute of contractile fibers. While the physiologist is primarily interested in fibers of classes 2 and 3, the extensive work which has already been done on class 1 has served admirably as a basis on which to pattern the analysis of classes 2 and 3.

While the early work on tendon (see Katz[3]) was compatible with the view that this type of fiber conforms to the general type mentioned above, recent work from the laboratories of

Wyckoff[4] and of Clark[5] has brought evidence difficult to interpret on this basis. These studies reveal well oriented spacings as large as 432A *along the fiber axis*. This suggests that tendon may be made up of very large, well oriented protein molecules or micelles; the tendon might almost be thought of as a crystalline structure. Wyckoff and Corey[4] have even been able to reprecipitate with salts the collagen of rat tail tendon which had been dissolved in acetic acid, to form a "tissue" which when stretched and dried gave patterns identical with those of untreated tendon. To the biologist seeking a physical basis for structure building in cells and tissues, the possibility of such a crystalline array of giant protein molecules is highly suggestive, for from the time of the discovery of the cell, a century ago, leading investigators in almost every branch of experimental biology have found it necessary to postulate the existence of some kind of giant protein molecule or aggregate in protoplasm.

A possible alternative interpretation of the large meridional spacings in tendon is that this fiber consists of parallel bundles of indefinitely extended polypeptide chains along which amino acid residues are regularly repeated with a master period of the order of several hundred angstrom units. There is no direct evidence in support of such an interpretation; it is interesting, however, that geneticists have postulated the existence of some such a linear array of amino acid residues in the chromosomes, the master period corresponding to gene levels along the chromosome.

Another characteristic of protein fibers, investigated extensively by Astbury in the case of hair, is the tendency for the protein primary valence chains to be thrown into folds in planes parallel to the axis of the fiber. This has been called the *alpha*-condition, as distinguished from the straight chain, *beta*-configuration.

Astbury and Dickinson[6] have shown that, like hair, muscle fibers must be composed of protein chains folded upon themselves as in the alpha-configuration. Moreover the diffraction pattern of contracted muscle is also that of an alpha-fiber, from which Astbury concludes that contraction consists of a reversible super-folding of the chains in analogy to the phenomenon of supercontracture in hair. It is to be hoped that further analysis along these lines promised shortly by Astbury will reveal the more intimate details of the contractile process.

Leaving the x-ray analysis of animal fibers and turning to the polarized light method we find that all protein fibers, with one known exception, show both positive intrinsic and positive form birefringence. They are composed of uniaxial crystallites with the optic axes paralleling the fiber axis. Among these we may list collagen fibers as in tendon, skin, etc., all contractile fibers, such as myofibrils (of smooth, striated and cardiac muscle), cilia, the motile tails of sperm cells, stereopods of protozoa, and the spindle and astral fibers of dividing cells (see Schmidt[7]).

Much interest attaches to the case which forms an exception to the above mentioned general rule, namely fibers composed of nucleoprotein. Artificially spun fibers of nucleoprotein show weak positive form birefringence like other protein fibers, presumably due to the longitudinal orientation of the protein chains. They also show relatively strong negative intrinsic birefringence due presumably to the condensation on the protein chains of nucleic acid groups oriented perpendicular to the protein chains. The sign of birefringence of the nucleoprotein fiber will, be determined, therefore, by the relative importance, under any given condition, of the form and the intrinsic factors. These considerations explain the birefringence of chromosomes, which, in the closely packed state found in sperm heads, show strong negative birefringence but in the more expanded condition as seen in cell division, show weak positive birefringence. Particularly instructive for the analysis of chromosome ultrastructure are the giant banded chromosomes of insect salivary glands. In Chironomus, W. J. Schmidt[8] finds the chromatic bands negatively birefringent with respect to the length of the chromosome, the alternate regions between these bands being isotropic.

Since such negative birefringence is doubtless due to nucleoprotein, the polarized light studies confirm the work of Caspersson[9] based on ultraviolet absorption spectra, that the nucleic acid residues are localized preponderantly in the chromatic bands. The work of Caspersson, however, leads one to the view that the chromo-

some is essentially a protein fibrous structure, the chromatic bands being regions in which relatively high concentrations of nucleic acid are condensed upon the protein chains. One might therefore expect the regions between the chromatic bands to show weak positive birefringence. Dr. Heys and I (unpublished) have observed such effects in the case of the giant chromosomes in Drosophila salivary glands, although it is possible that longitudinal tension incident to the preparation of the chromosomes may have been partially responsible for the phenomena.

Since a given chromosome in a given species apparently always shows the same pattern of banding from one cell to the next and since these bands have been associated with definite gene levels by geneticists, a careful analysis of chromosome ultrastructure by polarization optics may throw important light on the physical basis of heredity.

FIG. 4. Negative polarization cross in the cell sheath and nuclear membrane of a nerve cell from the ventral cord of the leech. The cell is immersed in Ringer solution. The axon emerging from the cell shows positive birefringence with respect to its length. Original photograph taken by Dr. P. Chinn.

One of the most highly expanded, highly solvated systems of tissue fibrillae is that found in the aqueous core of the nerve fiber, e.g., the neurofibrils of the axis cylinder. In fixed and stained preparations the axis cylinder may be fairly packed with clear-cut fibrillae oriented parallel to the fiber axis. In the fresh condition, however, the axis cylinder is optically empty, no trace of any fibrillae being detectable either with the ordinary microscope or with the ultramicroscope, and histologists and physiologists have been divided in opinion as to whether these fibrillae exist in the living nerve fiber or are merely artifacts of fixation. The polarized light method has assisted considerably in the clarification of this problem. The fresh axis cylinder shows weak positive birefringence which, analysis shows, is due primarily to the form factor but to some extent also to intrinsic birefringence typical of true fibers. Application of the Wiener theory in a somewhat modified form (see equations, Fig. 2) suggests that a very small fraction of the solid material of the axis cylinder is present in an oriented state in the fresh fiber but that the copious fibrillation typical of fixed tissue is due to oriented adsorption of axis cylinder material upon the rather tenuous lattice work present in the fresh fiber, Bear, Schmitt and Young.[10]

2. *Lamellar (Membranous, Film-Like) Orientation*

Almost all of our information about the tendency of protein particles to aggregate in the form of sheets or layers comes from polarized light studies, although recent work on the nerve sheath has added evidence from x-ray diffraction. The chief evidence of the lamellar orientation of the ultra-particles is the fact that the systems show negative uniaxial form birefringence. And in all of these cases the structure resembles a membrane or sheet when viewed in the living cell, or it displays evidence of lamellar structure in histological preparations.

The cell membrane and the nuclear membrane are among the best examples of this type of structure. Viewed in polarized light both the cell membrane and the nuclear membrane of living cells may display a negative polarization cross (Fig. 4). That this is due largely to platelet form birefringence is shown by the fact that if the cell be immersed in media of refractive indices higher than that of water, such as methyl alcohol, xylol, benzene, etc., the birefringence is markedly decreased (see curve 1, Fig. 5). It will be noted that these fluids are efficient lipoid solvents. If instead of these we choose media such as aqueous solutions of glycerine, urea, sugar, etc., which also have relatively high refractive indices but which are not lipoid solvents, the birefringence not only decreases but actually reverses sign

(see curve 2, Fig. 5). These facts can be interpreted only on the assumption that the membrane is built of a complex of lipoids and protein. Both of the components are uniaxial with optic axes perpendicular to the surface of the membrane. The lipoid component shows positive intrinsic birefringence while the protein component shows mostly negative form birefringence. The sign of birefringence of the membrane in the living cell will be determined, therefore, by the relative proportions of protein and lipoid. In the case of the cell and nuclear membranes the proportion of lipoid is so small that the protein characteristics predominate optically in the living cell and immersion methods are required to bring evidence of the presence of the oriented lipoid. The nuclear membranes of some cells such as nerve cells do not show the reversal of birefringence, hence contain very little lipoid (Chinn[11]); those of other cells, such as the starfish egg (unpublished observations made in collaboration with Dr. Chambers) have sufficient lipoid to show the reversal clearly. The duplex constitution of the cell membrane has been demonstrated in the case of red cell ghosts (Schmitt, Bear and Ponder[12]), and nerve cells (Chinn[11]).

Perhaps the best structure in which to study the lipo-protein organization of cellular sheaths and membranes is the axon sheath of nerve fibers. In this case nature has provided us with a graded series of sheath types from the so-called unmyelinated fibers of invertebrates to the heavily myelinated sheaths of the vertebrates (Schmitt and Bear[13]). In the former type there is so little oriented lipoid in proportion to protein that it can be detected only with difficulty, even by polarized light methods, while in the latter the protein form birefringence is insignificant in comparison to the intrinsic birefringence of the lipoid. Being comparable in certain respects with the cell and nuclear membrane, the myelin sheath of the vertebrate nerve fiber is particularly suitable for a detailed analysis of the molecular organization of lipo-protein complexes as found in cells and tissues generally. Moreover, since the myelinated nerve fiber lends itself readily to analysis by means of x-ray diffraction, detailed information regarding its molecular organization can be obtained.

The results of the analysis (Schmitt, Bear and Clark[14]) may be summarized briefly as follows. The sheath may be thought of as possessing a concentric cylindrical layer structure in which the distance in a radial direction between the consecutive layers is 171A. Considering a single layer we find that the lipoid molecules are constrained to remain within their own layer, are oriented with their long paraffin chains extending radially but have perfectly random orientation,

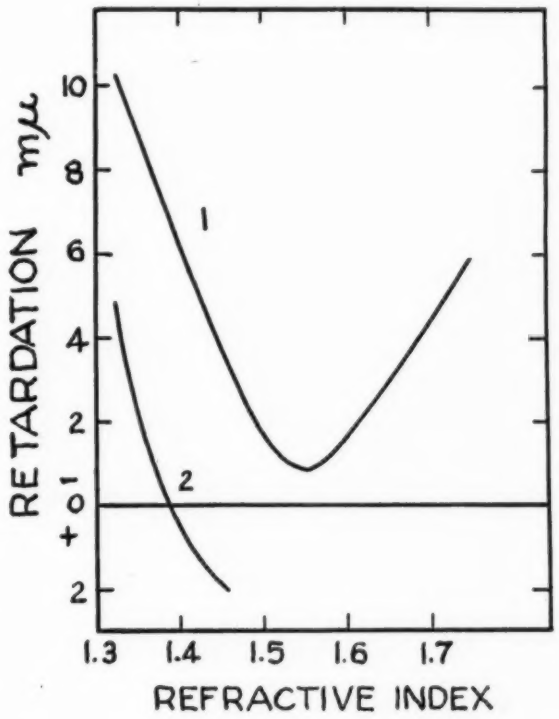


FIG. 5. Birefringence curves of the sheath of fixed frog spinal ganglion cells (data from Chinn[11]). Curve 1 is for a cell which had been extracted with organic solvents to remove the lipoids in the membrane before immersion in glycerine, sucrose and glucose (to obtain the form birefringence curves). Curve 2 is for a similar cell from which the lipoids have not been previously extracted. Reversal of birefringence in media of high refractive index indicates the presence of oriented lipoids in the membrane.

in the plane of the layer. This has been deduced from the fact that the periodicities in tangential directions are of the type given by true liquids while the interlayer periodicities are more nearly of the crystalline type. We appear to be dealing with layers having the properties of smectic fluid crystals wrapped about a cylinder.

It has long been known that lipoid molecules

tend to associate in double layers to form smectic fluid crystals, such, for example, as give rise to myelin forms. The possibility existed, therefore, that the 171A spacing in the nerve sheath is not unique for this structure but results from the tendency of its lipoids to build multi-layered films. To test this possibility patterns were obtained from typical nerve lipoids (phosphatids, cerebrosides and sterols) both in the moistened and in the dry condition. The spacings obtained were those to be expected from double layers of these molecules, e.g. the largest spacing was that from cerebroside which was approximately 80A. It is apparent, therefore, that the 171A spacing is not due alone to a multi-layered packing of lipoids.

A solution of the problem was suggested by a consideration of the polarized light data which show that beside the lipoid molecules, the sheath contains protein material arranged in concentric layers (see Schmidt,[15] Bear and Schmitt[16]). The diffraction data were then reexamined to test the hypothesis that the protein forms an integral part of the fundamental period of 171A, being represented in fact by one or several layers of protein intercalated between, and perhaps exerting a binding influence upon, two double layers of lipoid. If this is true there would be a space of 10–40A. available for this protein layer depending on the average figure used for the thickness of the lipoid double layers. Consideration of the intraperiod structure on the basis of the relative intensities of the orders of the fundamental spacing of the diffraction photograph, four of which are clearly measurable, has supported this view. It is of course impossible either from the optics or the diffraction data to say anything about the arrangement of the protein molecules within these concentric layers. They may be arranged in a two dimensional grid after the manner suggested by the cyclol theory of Wrinch[17] or they may consist of particles or molecules which are asymmetric and which have their long dimensions lying in the concentric levels.

The polarized light studies show that cell and nuclear membranes as well as the nerve sheath are constructed in general of an intermixture of lipoid and protein molecules oriented with long axes mutually perpendicular, e.g., perpendicular and parallel, respectively, to the surface of the membrane. The x-ray diffraction analysis of the nerve sheath, however, reveals the specific manner in which the lipoid and protein components are arranged. The sheath appears to be built of a regular repetition of concentric layers, the unit aggregate in the radial direction being 171A in thickness and consisting of a layer of protein intercalated between two double layers of lipoid molecules. The close analogy of this structure with that of the built-up lipo-protein films described by Dr. Langmuir is obvious. The similarity is in fact so striking that one is led to attempt, on the basis of the conditions necessary for producing such built-up films artificially, to visualize the processes in developmental mechanics by which the nerve sheath and other lipo-protein membranes are laid down in the organism. It is perhaps not too much to predict that a study of films artificially built of lipoids and proteins will go far towards explaining not only the molecular architecture of the normal nerve sheath but also certain of the specific properties of permeability and electrical polarizability of this structure. Experiments along these lines are now in progress, the films being built of lipoids known to be present in the nerve sheath rather than of the simple fatty acids used by Langmuir in laying the groundwork for the technique.

Bibliography

1. O. Wiener, "Die Theorie des Mischkörpers," Abh. sächs Ges. Wiss. math. phys. Kl. **32**, 509 (1912).
2. D. Jordan Lloyd, "Colloidal Structure and its Biological Significance," Biol. Rev. **7**, 254 (1932).
3. J. R. Katz, *Die Röntgenspektrographie als Untersuchungsmethode* (Berlin, 1934).
4. R. W. G. Wyckoff and A. B. Corey, "X-Ray Reflections of Long Spacing from Tendon," Science **82**, 175 (1935). "X-Ray Diffraction Patterns from Precipitated Connective Tissue," Proc. Soc. Exp. Biol. and Med. **34**, 285 (1936).
5. G. L. Clark and J. A. Schaad, "X-Ray Diffraction Studies of Tendon and Intestinal Wall Collagen," Radiology **27**, 339 (1936).
6. W. T. Astbury and S. Dickinson, "α–β Intramolecular Transformation of Myosin," Nature **135**, 95 (1935). "α–β Transformation of Muscle Protein *in situ*," Nature **135**, 765 (1935).
7. W. J. Schmidt, "Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma," Berlin, 1937.

8. W. J. Schmidt, "Doppelbrechung der Chromosomen in den Speicheldrüsenkernen der Chironomuslarven," Naturwiss. **25**, 506 (1937).
9. T. Caspersson, "Ueber den chemischen Aufbau der Strukturen des Zellkernes," Skand. Arch. f. Physiol. **73**, suppl. 8, 151 (1936).
10. R. S. Bear, F. O. Schmitt and J. Z. Young, "The Ultra-Structure of Nerve Axoplasm," Proc. Roy. Soc. **B123**, 505 (1937).
11. P. Chinn, "On the Birefringence of Nerve Cells," Am. J. Physiol. **119**, 31 (1937). Complete paper in press.
12. F. O. Schmitt, R. S. Bear and E. Ponder, "Optical Properties of the Red Cell Membrane," J. Cell. and Comp. Physiol. **9**, 89 (1936).
13. F. O. Schmitt and R. S. Bear, "The Optical Properties of Vertebrate Nerve Axons as Related to Fiber Size," J. Cell. and Comp. Physiol. **9**, 261 (1937).
14. F. O. Schmitt, R. S. Bear and G. L. Clark, "X-Ray Diffraction Studies on Nerve," Radiology **25**, 131 (1935). Second paper in press.
15. W. J. Schmidt, "Doppelbrechung und Feinbau der Markscheide der Nerven fasern," Z. Zellf. u. Mik. Anat. **23**, 657 (1936).
16. R. S. Bear and F. O. Schmitt, "Optical Properties of the Axon Sheaths of Crustacean Nerves," J. Cell. and Comp. Physiol. **9**, 275 (1937).
17. D. Wrinch, "The Cyclol Hypothesis and the Globular Proteins," Proc. Roy. Soc. **A161**, 505 (1937).

As scientists we are inheritors of a noble and ethical tradition; we are the successors of men who loved truth and justice and their fellow-men more than fame or fortune or life itself. The profession of the scientist, like that of the educator or religious teacher, is essentially altruistic and should never be prostituted to unethical purposes. To us the inestimable privilege is given to add to the store of knowledge, to seek truth not only for truth's sake but also for humanity's sake, and to have a part in the greatest work of all time, namely, the further progress of the human race through the advancement of both science and ethics.—EDWIN GRANT CONKLIN

Here and There

Physics in the Automotive Industry

One of the most important meetings in the field of applied physics for this year will be held March 14 and 15 at Ann Arbor, Michigan. This meeting is concerned with the applications of physics in the automotive industry and is sponsored jointly by the American Institute of Physics and the University of Michigan. The papers scheduled at the present time are as follows:

Developments in the Automotive Industry Delayed by Want of Knowledge of Fundamental Principles. K. T. KELLER, *President, Chrysler Corporation*
Scientific Training and Its Relation to Industrial Problems. C. F. KETTERING, *Vice President, General Motors and Director, General Motors Research Laboratory*
What Physics and Physicists Can Do for the Automotive Industry. LYMAN J. BRIGGS, *Director, National Bureau of Standards*
The Nature of the Solid State. F. SEITZ, *Research Laboratory of General Electric Company*
Thick Film Lubrication in Journal Bearings. M. MUSKAT, *Chief of Physics Division, Gulf Research and Development Company*
Type of Physicists the Automotive Industry Needs. J. S. THOMAS, *President, Chrysler School of Engineering*
Some Factors Influencing the Flow of Physics into Industry. E. J. MARTIN, *Research Laboratory of General Motors Corporation*
Physical Problems of Noise Measurement. P. HUBER, *Proving Grounds of General Motors Corporation*
Automotive Acoustics and Electrical and Acoustical Analogies. F. A. FIRESTONE, *University of Michigan*
Studying Engine Combustion by Physical Methods. L. WITHROW AND G. RASSWEILER
A Few Instruments Used in Automotive Research. E. J. MARTIN, *Research Laboratory of General Motors Corporation*

As an added attraction an inspection tour of the physics laboratory at the University of Michigan will be planned. The facilities of the laboratory for research and instruction will be shown with particular emphasis on the researches that are now going on and that have been carried out on problems of industrial physics.

The arrangements at the University of Michigan are in charge of Professor O. S. Duffendack.

★

AAAS Prize

At the Indianapolis meeting of the American Association for the Advancement of Science the $1000 prize, given annually for an "outstanding" paper presented at the meeting, was awarded to Dr. Philip R. White, of the department of animal and plant pathology of the Rockefeller Institute for Medical Research at Princeton, N. J., for a paper presented before the Section for the Botanical Sciences and the Botanical Society of America entitled "Root Pressure—an Unappreciated Force in Sap Movement." The first Theobald Smith award in medicine, established by Eli Lilly and Company, was given to Dr. Robley D. Evans, of the Massachusetts Institute of Technology, for his address entitled "Biophysical Studies of Chronic Radium Poisoning."

American Physical Society

Dr. Lyman J. Briggs, director of the National Bureau of Standards, was elected president of the American Physical Society at the Indianapolis meeting. Other officers elected were: Dr. John T. Tate, of the University of Minnesota, vice president; Professor W. L. Severinghaus, of Columbia University, secretary, and Dean George B. Pegram, of Columbia University, treasurer.

★

New Appointments

Professor P. M. S. Blackett, of Birkbeck College, University of London, has been appointed to succeed Professor W. L. Bragg in the Langworthy chair of physics at the University of Manchester.

Science, December 31, 1937

★

Dr. Allen Goodrich Shenstone has been promoted to the 1909 professorship of physics at Princeton University.

★

Dr. Ralph W. G. Wyckoff, of the department of animal and plant pathology of the Rockefeller Institute of Medical Research at Princeton, N. J., has accepted a position with the Lederle Laboratories at Pearl River, New York.

★

Dr. C. G. Darwin has been appointed president of the Mathematical and Physical Sciences section of the British Association for the Advancement of Science.

★

Congressional Medal

A Congressional Medal "for scientific accomplishment unequalled in polar exploration" was presented to Dr. Thomas C. Poulter, director of the research foundation at Armour Institute of Technology, at a dinner given in his honor on January 19. Dr. Poulter was senior scientist and second in command to Rear Admiral Richard E. Byrd on the second Byrd Antarctic expedition, which left the United States in October, 1933, and returned in May, 1935. The medal was presented by Governor Horner, and Rear Admiral Byrd spoke.

Science, January 14, 1938

New Spencer Lens Plant

The Spencer Lens Company announces that it has completed negotiations for the purchase of twenty-five acres in Cheektowaga, a suburb of Buffalo. It is expected that a new production unit will be built there soon, and other units will be constructed as the need arises.

★

Thomas A. Edison Medal

Dr. Gano Dunn, president of the J. G. White Engineering Corporation of New York for twenty-four years, has been awarded the Thomas A. Edison Medal of the American Institute of Electrical Engineers for 1937. The award is made "for distinguished contributions in extending the science and art of electrical engineering, in the development of great engineering works and for inspiring leadership in the profession." The presentation ceremony was held January 26 in connection with the annual four-day winter convention of the institute in the Engineering Societies Building, New York City.

Science, December 24, 1937

★

Western Society of Engineers Award

The Washington Award of the Western Society of Engineers for 1938 will be presented to Dr. Frank B. Jewett, President of the Bell Telephone Laboratories. This award is given for "the engineer whose work has contributed most to human progress."

★

Appointments Available

The Armour Institute of Technology has announced the following scholarships, fellowships and assistantships, available in their graduate division, for the year 1938–1939.

1. Scholarships—available only to students of good scholastic qualifications. Stipend—$300–$400 a year.
2. Fellowships—available only to students of excellent scholastic qualifications. Stipend—$400–$900 a year. Fellowships may be on a full-time or half-time basis.
3. Teaching and Research Assistantships—available only to students whose recommendations imply unusual success in the graduate field. Stipend—on a half-time basis—$800 for the first year, $900 for the second year.
4. Industrial Fellowships and Assistantships—available to students who show particular promise in industrial design or research work. Stipend—on a full-time cooperative basis—$1000–$1200 a year.
5. Post-Doctorate Fellowship in the Research Foundation. Probable stipend—$1600–$2000.

Payment of tuition of $150 per year on a half-time basis or $300 per year on a full-time basis is required of all graduate students. These appointments apply to the departments of civil engineering, electrical engineering, mechanical engineering, chemical engineering, physics and mathematics.

Application should be made before February 20, 1938 to the Dean of the Graduate Division, Armour Institute of Technology, Chicago, Illinois.

Institute of the Aeronautical Sciences

The Institute of the Aeronautical Sciences held its sixth annual meeting at Pupin Physics Laboratories, Columbia University, New York on January 24–27. Technical sessions were held on the general subjects of Structures, Instruments, Airplane Symposium and Discussion, Rotary Wing and Lighter-Than-Air Aircraft, Meteorology, Power Plants and Propellers, Aerodynamics, Air Transport and High Altitude Flight.

★

Meeting of the Intersociety Color Council

The Intersociety Color Council, an organization of national societies and of individual members interested in the standardization, description and specification of color, is holding its annual meeting at the Electrical Testing Laboratories, New York, on February 24. The program is as follows:

1st Session at 9:30 A.M.—Annual Business Meeting

Luncheon at 1:00 o'clock

2nd Session at 2:00 P.M.—Technical

1. **History of the Design of the Spectrophotometer.** A. C. HARDY, *Massachusetts Institute of Technology, Boston, Mass.*
2. **Construction of the Spectrophotometer.** J. L. MICHAELSON, *General Electric Company, Schenectady, N. Y.*
3. **Calibration and Operation of Spectrophotometer.** K. S. GIBSON, *National Bureau of Standards, Washington, D. C.*
4. **Application of Spectrophotometric Data to the Printing Industry.** C. E. FOSS, *International Printing Ink Corp., New York, N. Y.*
5. **Identification of Gelatins for Theatrical Lighting.** D. B. JUDD, *National Bureau of Standards, Washington, D. C.*

Supper at 6:30 P.M.

3rd Session at 8:00 P.M.—Popular

1. **Psychology of Color.** F. L. DIMMICK, *Hobart College, Geneva, N. Y.*
2. **Color in Interior Design.** ETHEL LEWIS, *Associate Editor, Interior Design and Decoration, New York, N. Y.*
3. **Solar Corona, Painting and Description.** C. BITTINGER, *Artist, Washington, D. C.*
4. **Colored Motion Pictures of the Growing of Crystals Under Polarized Light.** L. A. JONES, *Eastman Kodak Company, Rochester, N. Y.*

★

Calendar of Meetings

American Association for the Advancement of Science
Ottawa, Canada, June 27–July 2, 1938

American Physical Society
New York, N. Y., February 25–26, 1938
Washington, D. C., April 28–30, 1938
Metropolitan Section
New York, N. Y., March 25, 1938
Toronto, Canada, June 25–26, 1938

Optical Society of America
New York, N. Y., February 25–26, 1938

★

Necrology

Science reports that Simeon E. Boomer, for twenty-five years head of the department of physics and astronomy at the Southern Illinois State Normal University, died on January 3 at the age of sixty-three years.

Innovations in Instruments

New G-E Dew-Point Potentiometer

Dry gases are required in many industrial processes. One use is as protective atmosphere in the annealing of high carbon steels. Moisture in the furnace atmosphere is detrimental in this process because it decarburizes the steel. A new portable instrument for determining the moisture content of gases is announced by the General Electric Company.

A small stream of the gas is conducted through a compartment of the instrument. There it comes into contact with a thin metallic mirror to which a thermocouple is attached. This thermocouple is connected to a potentiometer and to an indicating meter, the scale of which is calibrated in degrees of temperature. A window is provided in the compartment so that an observer can see the mirror, and a lamp is provided so that the instrument can be used in dark places or at night.

The mirror is cooled by a small stream of cooling gas directed against its back through a suitable nozzle. Compressed gas, such as carbon dioxide, is convenient for this purpose since it may be obtained in small, portable, storage cylinders. For lowest dew-point measurements, moisture-free carbon dioxide should be obtained. The lower temperatures are required when the moisture content of comparatively dry gases is to be measured.

In taking a measurement, the operator increases the flow of cooling gas against the mirror until a spot of moisture condenses on its surface. Slight adjustments of the cooling effect will then cause the alternate evaporation and condensation of moisture on the mirror. At that point, the dew-point temperature can be read directly on the meter after first balancing the galvanometer. This balancing is accomplished by turning a knob until the galvanometer is at zero. The scale range of the dew-point potentiometer is from 130°F to —40°F.

★

Fluorescent Chalk

A fluorescent chalk which glows with a strong green light and is visible at a distance has been developed recently by the Westinghouse Electric and Manufacturing Company. This new material appears and marks like ordinary chalk under normal light. It glows in the dark when irradiated with ultraviolet rays such as from a sunlamp enclosed in a black globe. It provides a new tool for the lecturer who, during the showing of stereopticon or moving pictures, wishes to put a visible written message on the blackboard for his audience.

Vacuum Cement

A cement for vacuum furnaces which does not become brittle, adheres to any surface and can be reused indefinitely, has been developed by the Westinghouse Electric & Manufacturing Company recently. This cement is solid at room temperature, but at higher temperatures becomes increasingly softer. It does not deteriorate in any way with use, so it can be reclaimed, the fragments melted together and used again and again without limit. This cement is highly tenacious and will stick to rough or smooth metal, glass and quartz surfaces to form vacuum-tight joints.

In applying it to a vacuum furnace, the cement is smeared over the joints of the cap closing the inner chamber of the furnace. A torch is used to make the compound flow readily and a heated putty knife to spread it. This cement can also be used as a paint by using carbon tetrachloride as a solvent. In this form it can be painted on and a layer of the desired thickness built up.

★

New Thermionic Amplifier

The Leeds and Northrup Company announces a new thermionic amplifier which is inserted between a potentiometer and a suitable external galvanometer. Potentiometer measurements may then be made in the usual way. The

measurements are true null measurements. The only current drawn from the measured circuit being the minute electron tube controlled from it. The control current may be adjusted to be less than 10^{-14} ampere at balance, and when so adjusted the error caused by this current is only 1.0 millivolt in a 100,000 megohm circuit. For circuits of lower resistance this error is proportionately less.

Glass Marking Inks

Inks for marking on glass, porcelain and metal have been developed by the Westinghouse Electric & Manufacturing Company. Glass marking inks in both black and white, which adhere to a surface when applied with a steel pen are available. The composition of these inks is such that it will not interfere with the vacuum conditions in a vacuum chamber where it may be used. If set by the application of a moderate temperature they cannot be removed completely by ordinary scraping. Both of these inks can be used on glass beakers, porcelain surfaces and thermometers, as well as for decorative effects.

A silver monogram ink, used primarily for the "W" monogram on Mazda lamps, has also been developed. This ink may be applied with a rubber stamp, but in order to fuse the metallic silver deposit into the glass, it must be dried by the application of heat. A black monogram ink, drying in two minutes, and which can be applied with a rubber stamp is also on the market.

★

RCA New Vacuum Tubes

Type		
1G5-G	Power amplifier pentode	2.0 volt filament
6AC5-G	High-mu power amplifier triode	6.3 volt heater
6C8-G	Twin triode amplifier	6.3 volt heater
6F8-G	Twin triode amplifier	6.3 volt heater
6G6-G	Power amplifier pentode	6.3 volt heater
6U7-G	Triple-grid super-control amplifier	6.3 volt heater
6Z7-G	Twin triode power amplifier	6.3 volt heater
6ZY5-G	Full-wave high-vacuum rectifier	6.3 volt heater
25A7-G	Pentode—rectifier	25.0 volt heater
6V6	Beam power amplifier	6.3 volt heater

★

Molybdenum Wire, Rod and Sheet

Molybdenum, widely used for parts of electronic tubes and lamps, has been announced by the Westinghouse Electric and Manufacturing Company in the forms of round wire and rod, and in sheets. The wires and rods are from one thousandth of an inch to three-quarters of an inch in diameter. The sheets are up to several inches in width and from 2½ to 30 thousandths of an inch thick. This product is unique in its resistance to high temperatures, at the same time being workable with relative ease.

★

Stopcock Greases

Special greases for use on glass cocks in vacuum lines have been developed by the Westinghouse Electric & Manufacturing Company. Four types of grease are announced. Stopcock grease No. 6 is for vacuum line stopcocks which may be subject to high temperatures. It is very viscous even at relatively high temperatures. No. 7 is for glass stopcocks in high vacuum exhaust systems. It will not readily release gases which would interfere with vacuum readings. No. 8 is for the lubrication of rubber hose connections to metal tips of vacuum exhaust pumps. Not only will it lubricate such a joint, but it will also assist in making it vacuum tight. No. 10 is recommended for general laboratory use. Its light consistency makes it most suitable for atmospheric pressure use and for easier manipulation during the winter months. It is particularly adapted to desiccator use.

★

Stainless Steel Thermometer

A new type of laboratory thermometer, provided with a dial-and-pointer scale encased in stainless steel, mounted on top of an 8-inch stainless steel stem, is being introduced by the Weston Electrical Instrument Corporation, Newark, New Jersey. The pointer is actuated by means of an internally balanced double coil of thermostatic bimetal sealed in the lower 1½ inches of the seamless stem. When the stem is immersed to a depth of 1½ inches in a liquid (3 inches in gas or vapor), the dial reads temperature values accurately without the necessity for stem correction.

Accuracy of the unit is guaranteed to ½ of 1 percent over the entire scale. Initial models of the unit are being offered in the following scale ranges: 0–220 deg. F, 50–300 deg. F, 50–500 deg. F, 0–100 deg. C, 0–150 deg. C.

★

Chromatic Stroboscope

A device called the Chromatic Stroboscope for the precise and rapid measurement of frequencies in the range represented by the piano keyboard has been developed by C. G. Conn, Ltd., Elkhart, Indiana. By a visual method, the deviation of a given tone from the equally tempered scale based on the standard A of 440 cycles per second may be read directly. The Chromatic Stroboscope may be used in piano and organ tuning, investigations of pitch, and musical instruction and demonstration.

Some of the New Books

Heat and Thermodynamics. MARK W. ZEMANSKY. Pp. 388+xii, Figs. 101, 15×23 cm. McGraw-Hill Book Company, Inc., New York, 1937. Price $4.00.

This book was designed as a text for students "who are in the first stage of preparation for a career in theoretical physics, theoretical chemistry, or engineering." It assumes a year each in general college physics and calculus. Additional mathematical treatments are developed as needed. "The first ten chapters deal with the fundamental ideas of heat, reversibility, entropy, etc., in a manner which strives at a compromise between rigor and simplicity. The remaining nine chapters deal with physical, chemical, and engineering application in sufficient detail to show how the general principles are applied to specific cases."

The first chapter relating to temperature discusses in some detail the theory of their measurement except for the very high and the very low. In two later chapters, the usual experimental phases of expansion, heat capacity, heats of change of state, conduction, radiation, ideal gas laws, which are commonly stressed in intermediate courses, are given treatments which are unusually brief though very good as far as they go. The chapters on work and the first and second laws are much more fully treated. As a text for an intermediate course in heat which is concerned with students many of whom will not take advanced work in physics (as stated, such was not the author's purpose), the emphasis on subject matter appeals to the reviewer as unsatisfactory. In many institutions the mathematical requirements will also be rather difficult to meet. For the student who goes on for advanced work, however, the book will probably be found highly satisfactory. There are many applications of thermodynamic theory, with emphasis on illustrative problems, to various branches of physics and chemistry. Specifically there are applications to steam engines, heat capacities, expansion, compressibilities, porous plug effect, black-body radiation, stretched wires, surface films, reversible cells, thermoelectricity, magnetism, production of extremely low temperatures, changes of phase, thermionics, diffusion, chemical equilibria, ideal gas reactions, heterogeneous systems.

The Nernst heat theorem, however, is not discussed. In contrast to many texts, the physical side has been kept abreast of the mathematical side of the subject. In this respect, the book is much more satisfying to the reviewer than any treatment he has found elsewhere.

Throughout the text the author employs a consistent set of symbols which is given in a table at the front. For those thermodynamic potentials which are variously named, he uses enthalpy, the term which is rapidly displacing the misnomer total heat, Helmholtz function, for the physicist's free energy, and Gibbs function for the chemist's free energy. Throughout the inexact character of the differential of heat change is indicated by Q with the d crossed, a much to be desired emphasis which will save the beginner several pitfalls. Throughout also references are made to quasi-static processes in order to emphasize that strictly processes cannot be described as successions of equilibrium states though they may be viewed as a succession of states which differ only infinitesimally therefrom. Numerous appropriate problems are found at the ends of the chapters. Diagrams of thermodynamic surfaces and tables and plates of experimental values are more common than in most texts on thermodynamics.

Unfortunately the author has not seen fit to number important equations. He has attempted so to write the book that such references are not much needed. He has not succeeded completely; moreover, he overlooked the convenience of reference that occurs when equations are numbered. It also seems unfortunate that two symbols θ and T should be used for the absolute temperature. It would seem that the only times when a distinction in thought is needed are when the porous plug effect and the foundations of the thermodynamic temperature scale are being considered, and here T and T^1 would have sufficed. Generally it seems to the reviewer that the author's policies of treatment of equations and quantities have been quite consistent and accurate. A few lapses were discovered such as in the first equation on p. 95 where 273.2 has been written in place of 273.2°K and on p. 250, Table XXXVI, where the uncertain deg./dyne/cm² has been written in place of $\frac{\text{deg.}}{dy/\text{cm}^2}$ or deg./(dyne/cm²).

I hope to use this book as a text.

A. G. WORTHING
University of Pittsburgh
Pittsburgh, Pennsylvania

Electricity and Magnetism. SYDNEY G. STARLING. Sixth edition. Pp. 630, Figs. 449, 14½×22 cm. Longmans, Green and Company, New York, 1937. Price $4.00.

With this the sixth edition of this text, long a leading standard in the advanced field of classical electricity and magnetic theory Starling's *Electricity and Magnetism* takes on a new dress. To accommodate a 12 point leaded type and at the same time avoid an increase in thickness, the page size is increased from 5″ × 7½″ to 5½″ × 8½″ which is standard for texts of this character. The change from 10 point solid to 12 point leaded type has entirely relieved the

congested appearance of the pages of the earlier editions thus giving the book a definite reading appeal. The use of bold faced article headings gives a clarity and an openness of presentation which has been definitely lacking heretofore.

The author's statement that no attempt has been made to produce a new book, or to make comprehensive changes in content or in form of presentation scarcely does the book justice. Omissions have been made of material which has come to assume a lesser importance in the light of more recent developments. Of these we may mention ships' compasses and electro-magnetization, earlier types of magnetic wave detectors, electric oscillators, wave meters and rectifiers; also methods of determination of M/C and V of the electron by energy of rays. Figures of the most recent designs of galvanometers, meters and box resistances have replaced those used in previous editions. Numerous additions bring the text in line with recent advances in the fields of cosmic rays, magnetic field effects, electron optics, heavy hydrogen, atomic structure, para-, dia-, and ferromagnetic theory, superconductivity, radioactivity and photoelectricity.

G. A. SCOTT
University of Pittsburgh

Booklets Recently Received

Jena Colored Optical Filter Glasses for Scientific and Technical Purposes. This booklet of 18 pages gives complete transmission data and charts on the varieties of Jena filter glasses. This booklet is obtainable from the Fish-Schurman Corporation of 250 East 43rd Street, New York City.

Jena^{er} Glas für die Optik. In this booklet are described the various forms in which optic glass is available as well as the refractive index and dispersion of various optic glasses. It is obtainable from the Fish-Schurman Corporation of 250 East 43rd Street, New York City. This same company offers a 16-page booklet on Jena glass for laboratory and industrial uses.

A Glossary of Terms. (28 pp.) second edition. This booklet is a glossary of terms relating to rubber, its uses and manufacture, embodying the advantages to be gained by the use of Factice for the practical rubber worker.

The Educational Focus. (22 pp.) Vol. IX, No. 1, January, 1938. This issue contains articles on "Splitting Hairs," "Dark Field Microscopy," "Regeneration in Hydra," "Hermann von Helmholtz, Pioneer in Physiological Research," and "Microscopic Masons."

General Radio Experimenter. (8 pp.) Vol. XII, No. 7, December, 1937. This issue is devoted to noise. Articles are "Noise and Its Reduction" and "Background Noise Corrections in the Measurement of Machine Noise."

Among our Contributors

R. E. Holzer

Dr. Robert E. Holzer was born in Portland, Oregon in 1906. He received his A.B. degree at Reed College in 1926, and his M.A. and Ph.D. degrees in physics at the University of California in 1928 and 1930. During the years 1931 to 1933, he was a National Research Council fellow in physics at the University of Chicago. He is now assistant professor of physics at the University of New Mexico.

J. F. Herrick

Julia F. Herrick received the Ph.D. in the field of biophysics from the University of Minnesota in 1931. She was head of the physics department of Rockford College for five years. She is a member of the American Physiological Society and of the American Physical Society and is associate in the division of experimental surgery and pathology and instructor in experimental medicine, the Mayo Foundation. Her outstanding studies have been on circulation of the blood.

E. J. Baldes

Edward J. Baldes received the Ph.D. in the field of physics from Harvard in 1924 and the same degree was conferred on him for work in the field of physiology by University College, London University, in 1936. He is associate professor of biophysics, the Mayo Foundation, on the staff of which he has served since 1924. He is a fellow of the Institute of Physics and the American Physical Society and a member of the American Physiological Society and the Society for Experimental Biology and Medicine.

Contributed Original Research

Experimental Analysis of Rein's Thermostromuhr for Small Flows*

J. F. Herrick, *Division of Experimental Medicine,*

Edward J. Baldes, *Division of Physics and Biophysical Research,*

and

F. P. Sedgwick, *Special Student, The Mayo Foundation, Rochester, Minnesota*

(Received November 18, 1937)

The experimental curve is determined which shows the relation between the flow of blood in cubic centimeters per minute and the deflection of the galvanometer in millimeters for a range of flows between zero and about 3 cc per minute. The curve is similar to that calculated by Burton for the Rein thermostromuhr and that experimentally obtained by Jongbloed and Noyons with their aerothermorheograph. The maximal deflection of the galvanometer occurs at a flow (Ringer's solution) of about 0.08 cc per minute and for smaller or greater flows the deflection decreases. Since the deflection of the galvanometer approaches zero for very small flows and becomes zero for cessation of flow, whereas it usually decreases for increase of flow, one must be careful to distinguish between a cessation of flow and an increase. Difficulty will not arise if sufficient time is allowed for the establishment of thermal equilibrium. Certain controversies have arisen because this particular limitation of Rein's thermostromuhr was disregarded. The method, as we use it, is wholly unsuited for indicating variations in blood flow which occur in less than sixty seconds. It may be very reliable for indicating changes in blood flow which occur over a longer period of time.

SEVERAL years' experience with Rein's thermostromuhr has demonstrated the consistent relation that exists between blood flow, measured in cubic centimeters per minute and the deflection in millimeters of the galvanometer in the thermocouple circuit. This relation may be described by means of a hyperbola for blood flows within the normal physiologic range. A typical hyperbolic curve is shown in Fig. 1.

A mathematic equation which expresses this relationship is $GV^N=K$ where G=deflection of galvanometer in millimeters, V=blood flow in cc per minute, and N and K are constants characteristic for each unit.

It is obvious from this equation that when the flow of blood is zero, the deflection of the galvanometer is infinite (∞). A consideration of the schematic diagram of the diathermy thermoelement, in Fig. 2, shows that the difference of temperature between the thermojunctions at a, will be zero when the flow of blood is zero. This, of course, assumes a truly symmetrical unit where both thermojunctions receive heat uniformly when there is no flow of blood. The deflection of the galvanometer is consequently zero.

This apparent discrepancy between the theoretical and practical considerations of the problem for zero flow can be explained by assuming that the actually observed points start to deviate from the theoretical hyperbola after a certain minimal flow and that these observed points lie on a curve which reflects back through the origin.

Shortly after our first publication concerning Rein's thermostromuhr, Burton, who has contributed an appendix to this paper, became interested in the modification of the thermostromuhr for the region of small flows. He found, from purely theoretical considerations that the maximal deflection of the galvanometer occurs

* Appendix by Alan C. Burton.

at a flow of 0.16 cc per min. when the internal diameter of the blood vessel is 2 mm. Because these deviations from the theoretical hyperbola took place at such small flows—flows that are outside those that usually occur physiologically

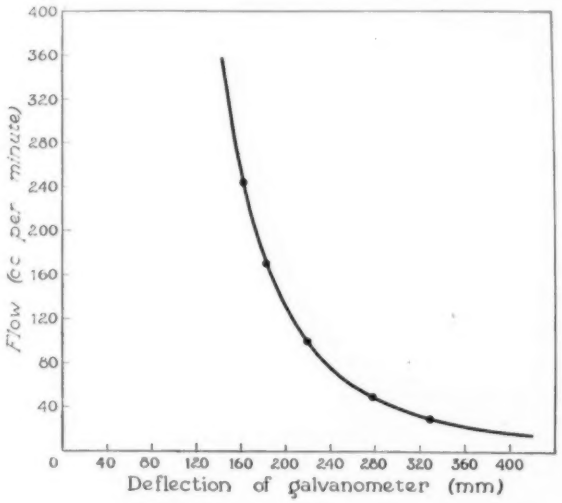


FIG. 1. Curve showing relation between the deflection of the galvanometer in the thermocouple circuit and the flow of blood in a given blood vessel for blood flows within the usual range. Theoretical curve: $G \times V^{0.335} = 1029.5$ (calculated from two arbitrary points). Dots indicate observed data.

—Burton did not publish his manuscript, simply stating that the deviation of the curve from the hyperbola in the region of small flows was only of academic interest.

The theory of the method in the region of small flows is of more than academic interest. It is probable that certain observations made when using the thermostromuhr have been misinterpreted owing to disregard of the behavior of the galvanometer at small flows. Anrep and his collaborators[1, 2] suggested that the diametrically opposite results obtained by Rein and by himself concerning changes in blood flow during tetanic contraction of skeletal muscle might be attributable to the response of the thermostromuhr to cessation of flow.

Jongbloed and Noyons[3] have applied the principle of Rein's thermostromuhr to the measurement of flow of gases. They called their apparatus the "aerothermorheograph." It consists of a small tube in the lumen of which a hot wire acts as a heater and replaces the platinum electrodes of the thermostromuhr. The thermojunctions lie on each side of the heater as in the Rein unit. The hot wire and the thermojunctions stand in the midst of the gas stream which flows through the tube. They found that the hyperbolic relation between the flow of gas, and the deflection of the galvanometer in the thermocouple circuit, obtains for large flows. However, for slow flows, the deflection of the galvanometer, having reached a maximum, decreases with decreasing flow. They found that values of flow between 200 liters per hour and 60 liters per hour fall on the theoretical hyperbola. However, when the flow becomes 50 liters per hour the experimental curve begins to deviate and finally reflects back through the origin. They predicted that the experimental curve of a liquid stromuhr would have the same general course. However, they stated: "*Dass der ganaue Verlauf der Kurve bei Flüssigkeitsdurchströmung sehr schwierig zu bestimmen ist, liegt vor allem daran, dass man es unter sonst gleichen Bedingungen im Wendepunkt-*

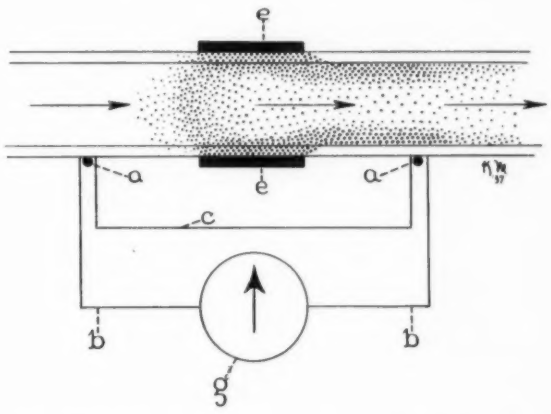


FIG. 2. Schematic drawing of a blood-flow unit diathermy thermoelement applied to a blood vessel: $e-e$, platinum electrodes; $a-a$, thermojunctions; c, constantan wire; b, copper wire; g, galvanometer. Arrows indicate direction of blood flow. Dots indicate heat distribution.

[1] G. V. Anrep, A. Blalock and Adli Samaan, "The Effect of Muscular Contraction upon the Blood Flow in the Skeletal Muscle," Proc. Roy. Soc. Med. §**B114**, 223–245 (1934).

[2] G. V. Anrep, S. Cerqua and Adli Samaan, "The Effect of Muscular Contraction upon the Blood Flow in the Skeletal Muscle in the Diaphragm and in the Small Intestine." Proc. Roy. Soc. Med. §**B114**, 245–257 (1934).

[3] J. Jongbloed and A. K. M. Noyons, "Zur Messung der Muskeldurchblutung während der Kontraktion mittels der Reinschen Thermostromuhr." Zeits. f. Biol. **96**, 554–562 (1935).

gebiet der Kurve und unterhalb desselben bei Gasdurchströmung noch mit sehr leicht messbaren Mengen zu tun hat, während bei Flüssigkeitsdurchströmung die Mengen dann schon so klein sind, dass eine genaue Messung kaum mehr möglich ist, wozu noch kommt, dass dann die Durchströmung wohl nicht mehr kontinuierlich vor sich geht, sondern Tropfen für Tropfen mit langen Zwischenpausen."

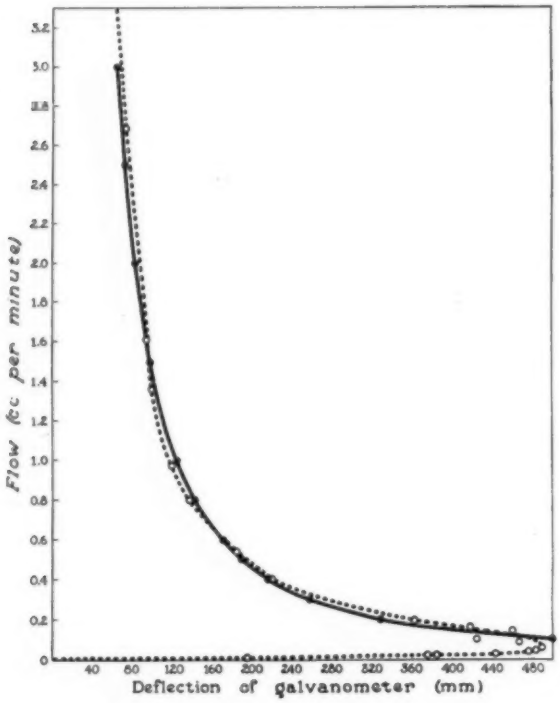


FIG. 3. Curve showing the relation between the deflection of the galvanometer in the thermocouple circuit and the flow of blood when the flows are very small. Black line indicates theoretical curve ($GV^{0.6061}=125$); dotted line, observed data.

Jongbloed and Noyons suggested a general equation for the complete experimental curve. Moreover they showed characteristic curves, both theoretical and experimental, accompanying transient cessations of flow and also brief increases of flow. These experiments indicate that Rein's[4] interpretation of an increase of flow at the beginning of tetanic contraction of skeletal muscle is not entirely justified. To quote the authors mentioned: "*Die Form des Initialgipfels der Blutdurchströmungskurve während der tetanischen Kontraktion des Skelettmuskels, wie sie erhalten wurde mit der Reinschen Stromuhr, lässt sowohl die Interpretation von vermehrter Durchströmung als auch von Stillstand der Durchströmung zu. Die Argumente, die gegen eine Vermehrung sprechen, scheinen aber genügend gewichtig, um den Reinschen Initialgipfel als Stillstand der Durchströmung aufzufassen.*"

As far as we knew, the experimental curve for Rein's liquid stromuhr in the region of slow flows never had been determined and we set out to determine it. The slow flows were obtained by inserting capillary tubes obtained from mercury thermometers into the circulatory system. The rate of flow was determined by observing the time for the meniscus of the fluid to travel between two calibrated points in pipets of various sizes. The factor of correction for each pipet was determined. Ringer's solution was used instead of blood. The nature of the curve could be determined easily with Ringer's solution and we did not feel that the additional difficulties involved when using blood justified its use. Fig. 3 shows the curve from zero flow to 2.69 cc per minute. The turning point of the curve is in the region of 0.08 cc per minute for Ringer's solution. The constants of the theoretical curve are computed from two arbitrary observations of the experimental data.

There appears to be a linear relation between the deflection of the galvanometer and the flow of Ringer's solution as the flow approaches zero.

The effects of certain drugs, for instance epinephrine, when given intravenously in large doses might be misinterpreted if one were not aware of the limitations of the method. The deflections of the galvanometer which sometimes occur immediately following injection of 0.1 cc of 1 : 1000 solution of epinephrine may be simulated when calibrating by momentarily clamping the rubber tubing distal to the diathermy thermoelement. The thermostromuhr indicates cessation of flow or very small flows by decreasing deflection. However, the effect of the drug subsides before the galvanometer has had time to reach its final deflection. The galvanometer deflects only partially and then returns to its former deflection. If these changes did not take place in less than

[4] Hermann Rein, "Die Thermo-Stromuhr. Ein Verfahren zur fortlaufenden Messung der mittleren absoluten Durchflussmengen in uneröffneten Gefässen in situ." Zeits. f. Biol. **87**, 394–418 (1928); *Abderhalden Handbuch d. Biol. Arbeit.* Vol. **8** (1928–1935), pp. 693–716.

one minute (the time required for the thermostromuhr to reach equilibrium) one might be justified in saying that an increase in blood flow resulted. However, the entire effect is over in less than thirty seconds. Since the action of the galvanometer can be reproduced completely in calibration by momentarily occluding the circulation distal to the unit, one is not justified in saying that an increase in blood flow occurred. There is little doubt that the epinephrine, in this particular instance, caused a transient, marked decrease of flow, perhaps to the point of cessation at some point distal to the position of the diathermy thermoelement.

The thermostromuhr of Rein is wholly unsuited for indicating changes in flow that occur in an interval of time less than that required for establishing thermal equilibrium.

Comment

The appendix by Alan C. Burton is a valuable supplement to our paper because it gives the theoretical justification for the curve which we have obtained experimentally—the curve which shows the relation between the deflection of the galvanometer in the thermocouple circuit and the flow of blood when the flow is very small. However, we wish to emphasize the fact that the theoretical treatment disregards certain important existing conditions and, therefore, arrives at equations which are only a first approximation.

We have studied units of the design suggested by Burton. The unit which we prefer at the present time is the one which has the thermojunctions symmetrically placed with regard to the heating element.

Appendix: Theory and Design of the Thermostromuhr

Alan C. Burton

Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia, Pennsylvania

As pointed out in the previous section, it is obvious that the curve of the deflection of the galvanometer against flow must pass through a maximum and reflect back to the origin. The situation is unsatisfactory until the underlying physical factors that determine this behavior of the curve are understood, in order that a prediction might be made as to the region of flow in which the deviation from the accepted type of formula will occur. The experimental work of the authors of the preceding section establishes that this region may be of physiological interest in a particular case, but theory should enable one to estimate the effect of size of the blood vessel and design of the various dimensions of the instrument upon the point of departure. It should be possible to design a thermostromuhr of this type to give the best shape of calibrated curve for the particular problem under investigation. The theoretical investigation that follows (originally made in 1934–1935) is a step in this direction.

The work of Baldes and Herrick[1] shows that the problem is a complicated one. The heating of the blood vessel wall cannot be disregarded in comparison with that of the blood itself. The flow is "streamline" except for high velocities where turbulence may occur. This means that the velocity is not constant across a cross section of flow, and in blood this results in a nonuniformity of the fluid itself, the cellular bodies being more concentrated in the axial region. A theoretical treatment taking all these factors into consideration would be unmanageable mathematically. The well-known method is therefore used in which only the factors essential to the point of interest, here the reflection of the curve back to the origin, are considered, and other complicating factors being "averaged." This simplification leads to a solution which is quite complicated enough.

This essential factor in the reflection of the curve to the origin is the *flow of heat by conduction in both directions of the blood stream* in contrast to the transfer by convection or transport of heat in the blood, which occurs in the direction of

[1] E. J. Baldes and J. F. Herrick, unpublished data.

flow only. At very small velocities of flow, the former dominates the picture and brings the temperature of the "preceding" and "following" thermocouples to equality. Using Fig. 2 of the preceding section, the problem may be set up in its simplest terms.

Assumptions

Let us suppose that heat is generated uniformly in the blood stream, at a rate h calories per second within the space enclosed by the electrodes e (Fig. 2), which have a length $2g$. Let the distance between the two thermocouples a be $2L$. Taking the midpoint of the electrodes as origin, one thermocouple lies at $X=-L$, the other at $X=+L$, the positive direction of X being in the direction of flow.

Our first assumption is then that between $-g<X<g$, h is constant, while outside this region h is zero.

Consider the blood lying between cross sections at distance X and $X+dx$. There will be a gradient of temperature across the section but it has an average temperature θ_x. Heat will leave the section through the wall to the surroundings at a rate proportioned to this average temperature, a rate which we write as $E(Sdx)\theta_x$, where S is the perimeter of the vessel, Sdx therefore the surface area, and E is a coefficient of heat transfer depending on the thermal conductivity of the wall. The fact that there is a gradient of velocity means that we cannot strictly consider that E is independent of the velocity of flow. As an approximation, however, we do so here.

Let A be the cross-sectional area of vessel, K the thermal conductivity of blood, ρ and s its density and specific heat. The various items of heat entering and leaving the section between X and $X+dx$ are given in Table I. V is the velocity of flow in cm/sec.

Table I.

Item	Heat Entering Section	Heat Leaving Section
By conduction in blood	$-KAd\theta/dx$	$-KA\,[d\theta/dx+d/d\theta(d\theta/dx)dx]$
By transport in blood stream	$V\rho sA\theta$	$V\rho sA\,[\theta+d\theta/dx\cdot dx]$
Heat produced	$hA\cdot dx$	
By loss to surroundings		$ES\cdot dx\cdot\theta$

If the steady state has been reached, heat entering must balance heat leaving the section. This gives

$$d^2\theta/dx^2+2a\cdot d\theta/dx+b\theta=c \qquad (1)$$

where

$$2a=-V\rho s/K,\quad b=-ES/KA,\quad \text{and}\quad c=-h/K.$$

The general solution of the equation is

$$\theta=c/b+M\epsilon^{\alpha x}+N\epsilon^{\beta x}. \qquad (2)$$

Where α and β have the values

$$\alpha=-a+(a^2-b)^{\frac{1}{2}},\quad \beta=-a-(a^2-b)^{\frac{1}{2}}. \qquad (3)$$

The values of M and N are found from the boundary conditions. Three regions have to be considered, $-\infty<X<-g$, where $h=0$, $-g<X<+g$ where h is constant, and $+g<X<+\infty$ where h is again zero. The three forms of Eq. (2) that apply to these regions must coincide in giving the same values of θ and of $d\theta/dx$ at the points where the regions meet, that is, at $X=+g$ and $X=-g$. The final result when the boundary conditions are satisfied is:

$$\begin{aligned}
&-\infty<X<-g,\ \theta=-\frac{2c}{b}\cdot\frac{\beta}{(\alpha-\beta)}\sinh(\alpha g)\cdot\epsilon^{\alpha x}\\
&-g<X<+g,\ \theta=\frac{c}{b}\left[1+\frac{1}{(\alpha-\beta)}\right.\\
&\qquad\left.\times(\beta\epsilon^{-\alpha(g-x)}-\alpha\epsilon^{\beta(g+x)})\right],\\
&+g<X<+\infty,\ \theta=-\frac{2c}{b}\cdot\frac{\alpha}{(\alpha-\beta)}\sinh(\beta g)\cdot\epsilon^{\beta x}.
\end{aligned} \qquad (4)$$

We are interested chiefly in the temperatures at the thermocouples, where $X=-L$ and $X=+L$:

$$\begin{aligned}
\theta_L&=-\frac{2c}{b}\cdot\frac{\alpha}{(\alpha-\beta)}\sinh(\beta g)\cdot\epsilon^{\beta L},\\
\theta_{-L}&=-\frac{2c}{b}\cdot\frac{\beta}{(\alpha-\beta)}\sinh(\alpha g)\cdot\epsilon^{-\alpha L}.
\end{aligned} \qquad (5)$$

Finally the deflection of the galvanometer is proportional to their difference.

$$G \propto -\frac{2c}{b}\cdot\frac{1}{(\alpha-\beta)}[\alpha \sinh(\beta g)\cdot\epsilon^{\beta L} - \beta \sinh(\alpha g)\cdot\epsilon^{-\alpha L}]. \quad (6)$$

Equation (6) must reduce to the usually assumed hyperbolic form for large values of V. In the expression (3) for α and β it can be shown that $\alpha \rightarrow V\rho s/K$ and $\beta \rightarrow 0$ for large velocities of flow. Eq. (6) then becomes

$$G \propto -\frac{2c}{b}\cdot\frac{\alpha\beta g}{\alpha-\beta}\epsilon^{+\beta L} \doteq \frac{2hg}{V\rho s}. \quad (7)$$

Eq. (7) is exactly what would result if we neglected conduction altogether. Since $(2hg)A$ is the total heat produced between the plates per second, while $(V\rho s)A$ is the total thermal capacity of the blood that has flowed through in this time, the rise of temperature is the ratio of these quantities.

Empirically an equation of the form (8)

$$G \propto (1/V^n) \quad (8)$$

is used to fit the data at large flows, where n is an index (less than unity) characteristic of the particular apparatus. An equation of this form may be made to fit the data given by Eq. (6) over a considerable range of the higher velocities. For the numerical example (data given below) this is shown by Table II, where n is 0.826. An equation of the form (8) therefore gives a fit up to slower flows than Eq. (7).

TABLE II.

Velocity cc/min.	0.3	0.5	1.0	1.5	2.0
G calculated Eq. (6)	0.167	0.117	0.066	0.047	0.035
G calculated Eq. (8)	.176	.116	.065	.047	.037

It should be noted (Fig. 3 of previous section), that an equation of the form (8) sometimes shows systematic deviation from actual data, indicating that the true equation is not of this form.

A second factor lowers the index of V in the empirical equation still more. When the gradient of velocity across the stream is taken into account, as the analogous gradient in the theory of "forced convection" (Boussinesq,[2] Russell[3]) has been, an index of 0.5 to 1.0 is encountered.

In Fig. 1, the deflection of the galvanometer in arbitrary units, is plotted against the velocity of flow, using Eq. (6), assuming particular numerical values of the constants given below:

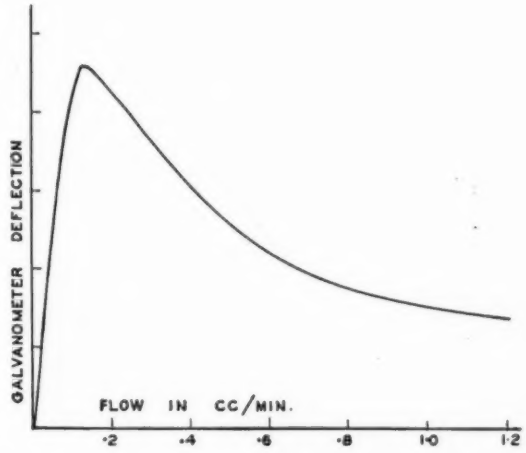


FIG. 1. Theoretical variation of deflection of galvanometer with flow for a given numerical case.

Diameter of blood vessel = 2 mm inside, 3 mm outside.
Density of blood, $\rho = 1.0$.
Specific heat $s = 0.8$.
Thermal conductivity = 0.0015 (that of water).

To obtain the value of E, a thermal conductivity of 0.0004 is assumed for the wall (an average value found for tissues), and the heat flow across this thickness of wall for a difference of temperature θ is equated to $ES\theta$, giving $E = 0.008$. For the dimensions of the stromuhr $g = 0.1$ cm, $L = 0.4$ cm is assumed.

The maximum deflection is reached in this case at a flow of 0.16 cc/min. That this is close to the value found experimentally by Baldes and Herrick may be considered of little significance. Since the thermal constants, especially the value of E, are very inadequately known, a correct order of magnitude is all that could be expected. The important consequence of the theoretical analysis is that the factors cooperating to give a maximum value of deflection may be understood. These appear clearly when the distribution of

[2] M. J. Boussinesq, Comptes rendus Acad. d. sc. **133**, 257–262 (1901).
[3] A. Russell, Phil. Mag. **20**, 591–610 (1910).

temperature in the apparatus is plotted for different velocities of flow from Eq. (4) (Fig. 2).

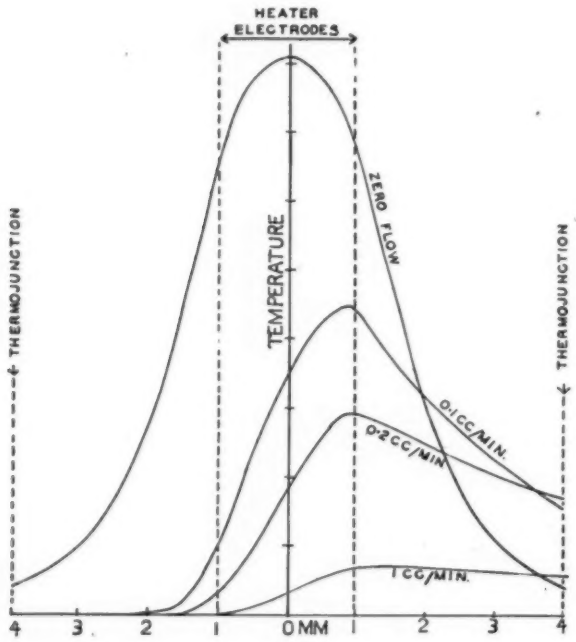


FIG. 2. Theoretical distribution of temperature in stromuhr for different velocities of flow.

With zero flow the distribution is of course symmetrical. As the flow increases two effects are seen.

(a) The distribution becomes asymmetrical, being deformed in the direction of flow. This tends to increase the temperature of the "following" over that of the "preceding" thermocouple and leads to the initial increase of deflection with flow.

(b) As flow increases the "amplitude" of the whole curve decreases throughout, and this factor, which decreases the deflection of the galvanometer with increasing flow, soon dominates the picture.

At flows even less than the critical flow for the maximum of the curve, the temperature rise of the "preceding" thermocouple may be neglected, so that down to almost zero flows the equation

$$G \propto -\frac{2c}{b}\cdot\frac{\alpha}{(\alpha-\beta)}\sinh(\beta g)\cdot\epsilon^{\beta L} \qquad (9)$$

suffices.

Unfortunately, differentiation of Eq. (6) or of (9) does not give a simple expression for the critical flow. Certain features of its dependence on the design of the apparatus may however, be deduced. The principle of similitude (dimensional analysis) tell us that the deflection must be a function of the quantity $(V\rho sy)/K$ where y is a linear dimension of the problem. Thus if all the dimensions are increased, as in a stromuhr on a larger vessel, corresponding effects on the curve will be seen at smaller velocities of flow. Since V is the linear velocity of flow (cm/sec.) this does not mean smaller volume flows, for volume/sec. $\propto V[y]^2$ i.e. $\propto [Vy]\cdot y$. Thus the critical volume flow will be greater within the bigger vessel.

Design of Stromuhr for Small Flows

If the thermocouples are moved closer to the heating electrodes, that is, L is decreased, the maximum occurs at lower flows, as can be seen from consideration of the two factors of Eq. (9) remembering that β is a minus quantity and decreases as V increases. Not much progress can be made here however. An alternative might be, for the investigation of very slow flows, to use the initial portion of the curve which is approximately linear, by making L large, that is, moving out the thermocouples. This presents practical difficulties and the ambiguity of interpretation of changes in deflection still remains.

For investigation with very small rates of flow the logical method would be to adopt the method used by Schmidt and Walker[4] in their modification of the stromuhr for direct current, namely, to place one thermocouple on the heating plates. Putting $X=0$ in (4) we have for the temperature here:

$$\theta=\frac{c}{b}\left[1+\frac{1}{(\alpha-\beta)}(\beta\epsilon^{-\alpha g}-\alpha\epsilon^{\beta g})\right] \qquad (10)$$

and if the other thermocouple "preceding" is sufficiently far removed we can neglect its rise of temperature except for almost zero flows. Using the same numerical values as before this is calculated and plotted in Fig. 3. The deflection is now not zero but maximal at zero flow. For comparison the curve of Fig. 2 (the symmetrical arrangement of thermocouples) is shown in

[4] C. F. Schmidt and A. M. Walker, "A Thermostromuhr Operating on Storage-Battery Current," Proc. Soc. Exp. Biol. and Med. **33**, 346–349 (1935).

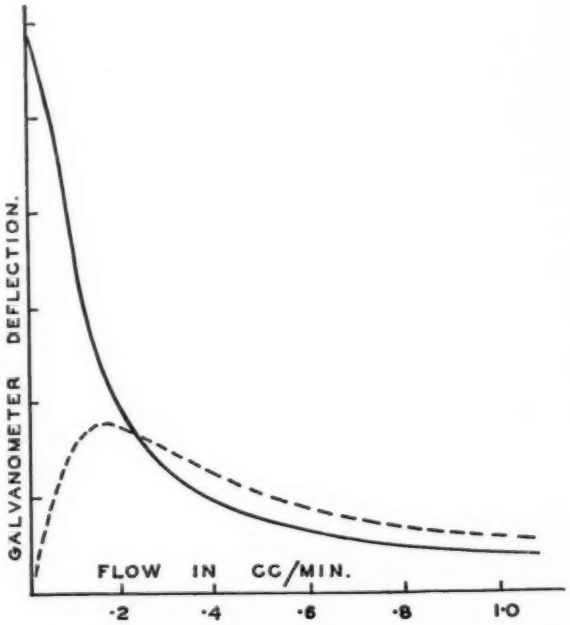


FIG. 3. Theoretical variation of deflection of galvanometer with flow in modified stromuhr with one thermojunction on heating electrode. The broken line shows the curve of Fig. 1 on the same ordinate scale.

broken lines on the same scale. For the same amount of heat production the slope of the curve is much greater in the region of slow flows, so that greater sensitivity might be obtained with no greater danger of heat injury to the tissue. This arrangement then offers great advantages over the usual one for this range of flow velocities. Some difficulties would arise from electrical pickup with asymmetrical arrangement of the thermocouples where high frequency current is used for heating, but none would be anticipated on this score with the direct current modification.

The foregoing analysis does not of course apply to the direct current instrument, where the blood is heated by conduction and convection from the heated electrodes. The problem becomes much more difficult to handle, as no longer may it be treated in one dimension only. In essentials however the results of such an analysis would be the same.

DESIGN FOR LARGE FLOWS

With either of the arrangements of thermocouples the equation for large flows approaches (7), where the galvanometer deflection becomes inversely proportional to the velocity of flow. This means relative insensitivity to changes in flow when the velocity is large. Little can be done in design to prevent this, as such behavior is inherent in the method. Changes in the dimensions of the instrument, such as L and g of the equations affect the curve appreciably only at low velocities. Increase in the heating current or in the length of the heating electrodes, preferably the latter, is the only recourse to increase the sensitivity for large flow velocities.

Condensation and Adsorption of Mercury Vapor

R. C. MASON*

Research Laboratories, Westinghouse Electric and Manufacturing Company, East Pittsburgh, Pennsylvania

(Received December 1, 1937)

The critical temperature of glass surface for condensation of mercury vapor as a function of density of atoms striking the surface has been found. The results indicate a large nucleus composed of many atoms is necessary for condensation to start; the heat of vaporization of an atom from the nucleus is 13.2 kcal. per mole. Some qualitative observations are reported, showing both rapid and slow adsorption of mercury atoms on glass and metal, and a considerable adsorption of mercury ions.

THE early observations of Wood[1] suggested that vapor atoms striking a solid were reflected if the solid were above a certain critical temperature, while they condensed if the solid were at a lower temperature. Later more careful analysis[2] developed the idea that each single

* Now at the University of Utrecht, Utrecht, Holland.

[1] Wood, Phil. Mag. **30**, 300 (1915); **32**, 364 (1916).

[2] Langmuir, Phys. Rev. **8**, 149 (1916); Proc. Nat. Acad. Sci. **3**, 141 (1917). Frenkel, Zeits. f. Physik **26**, 117 (1924).

atom striking the surface lingered a definite, short time if there were no neighboring atoms; but if two or more vapor atoms happened to lie side by side, their time of lingering might be much increased because of the strong forces between them. These two or more atoms of the vapor would then form a nucleus, upon which a visible condensate would grow. The time of lingering should depend on the temperature of the solid upon which condensation takes place: at a "critical" temperature, the probability of a condensation nucleus existing would become very great. The probability of formation of a nucleus should also be a function of the rate of arrival of atoms at the surface, hence dependent upon the vapor pressure. Experiments[3] using molecular rays show indeed a dependence of the critical temperature at which condensation appeared upon the density of the molecular beam, the variation being such as to indicate a nucleus of only two atoms necessary to start condensation.

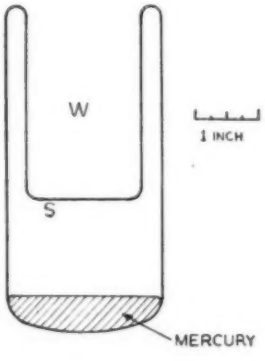


FIG. 1. Experimental tube for study of critical temperature for condensation of mercury on glass.

The previous work has been confined chiefly to cadmium, and, apparently the conditions for condensation of mercury on glass have not hitherto been determined quantitatively. In order to study this problem, a tube was constructed as sketched in Fig. 1. The tube of G702P glass was baked for 10 hours at 500°C, mercury was distilled in, then the tube was sealed off. The temperature of the target *S*, on which condensation was to be observed, was determined by liquid in the well *W*, while the vapor pressure of mercury and hence the rate at which atoms struck *S*, were controlled by a bath around the tube. The temperatures could be maintained constant easily to half a degree. With the outer bath at a chosen temperature, the target *S* was heated for some time in order to drive off any condensed mercury; then *S* was cooled to a definite temperature below that of the mercury. The temperatures were maintained for half an hour at the higher temperatures, or three hours at the lower; if no visible condensation had appeared, the experiment was repeated with greater temperature difference between target and pool, while if condensation did occur, a smaller difference was chosen, until the critical temperature at which condensation just appeared was found. Condensation first started at isolated points on the target; if larger temperature differences were employed, then the condensate became more general.

The vapor pressure of mercury in the tube, found from I.C.T. data for the temperature of the external bath, is plotted in Fig. 2 against the reciprocal of the absolute temperature of the target for which condensation occurred. Since half a degree variation in the baths was permitted, the pressures plotted correspond to the *highest* temperature of the mercury bath, while the reciprocal of the *lowest* temperature of the target was used. The upper points fall very closely along a straight line. The departures of the lower points may possibly be attributed to allowance of insufficient time for equilibrium to be established at these temperatures before judging that condensation would not be found. For comparison, the dotted line shows the saturation vapor pressure of mercury at the temperature given by the abscissa.

When condensation just occurs, the vapor pressure of the nuclei at which it starts must be slightly less than the vapor pressure of mercury in the gas space. The slope of the solid line in Fig. 2 thus gives the heat of vaporization of an atom from the condensation nucleus 13.2 kcal./mole. This line has so nearly the same slope as the one for the saturation vapor pressure of mercury that it is clear a very large nucleus, composed of many atoms—almost the equivalent of bulk mercury—must arise before visible

[3] Estermann, Zeits. f. Elektrochem. **31**, 441 (1925). Zeits. f. Physik **33**, 320 (1925). Cockcroft, Proc. Roy. Soc., London, **A119**, 293 (1928).

condensation can form. The horizontal separation of the two curves gives the temperature difference which must exist between condensing surface and the mercury which is supplying the vapor. The difference is extremely small—certainly not over 1° at the higher vapor pressures. These results say nothing about the speed at which condensation takes place, but merely represent equilibrium states.

Adsorption

As the density of mercury atoms adsorbed on a surface is important in determining the ultimate condensation on the surface, the adsorption on glass was studied in a tube which contained an ionization gauge for measuring the density of mercury atoms in the gas phase. A greaseless ground joint valve separated the tube from a mercury reservoir, so when mercury had been completely removed from the test tube by baking, by operation of the valve mercury vapor could be admitted and its adsorption studied by the ionization gauge. The many complicating factors described below prevented the exact determination of the amount of mercury adsorbed, especially the initial rapid adsorption, but a qualitative description of the results may be of interest.

1. Mercury atoms were adsorbed rapidly (within a few seconds) on the glass walls of the tube.

2. Mercury atoms were adsorbed on the cold metal parts of the ionization gauge. When the filament was heated, adjacent parts of the gauge also slowly warmed up, and from these, adsorbed atoms were driven off changing the vapor density in the tube.

3. It was repeatedly and convincingly demonstrated that mercury *ions* were strongly adsorbed on the metal and glass.[4] For instance, with an initial density of mercury atoms corresponding to *ca.* 1.6 microns, the ionization gauge was turned on. At first the vapor density rose, because of the effect described in 2, then fell over several minutes to about one-half the original value. Check tests with the filament heated but with no accelerating voltage on the gauge showed that the decrease in density occurred only when ions were being produced. In another experiment the ionization gauge was run for two hours with the valve between test tube and mercury reservoir open. The test tube was at 27° while the mercury supply reservoir was at 22.5°. The valve was then closed and the tube strongly heated. The density rose to almost three times the density with the valve open. While some of the mercury undoubtedly was adsorbed in atomic form, a considerable part must have been adsorbed as ions. Experiment shows the adsorbed ions may remain attached for many minutes at room temperature.

4. In addition to whatever rapid adsorption of mercury which may have taken place, mercury atoms were also slowly adsorbed, or "cleaned up," over long periods of time. A tube was sealed off containing mercury vapor at one micron pressure, plus an unknown amount

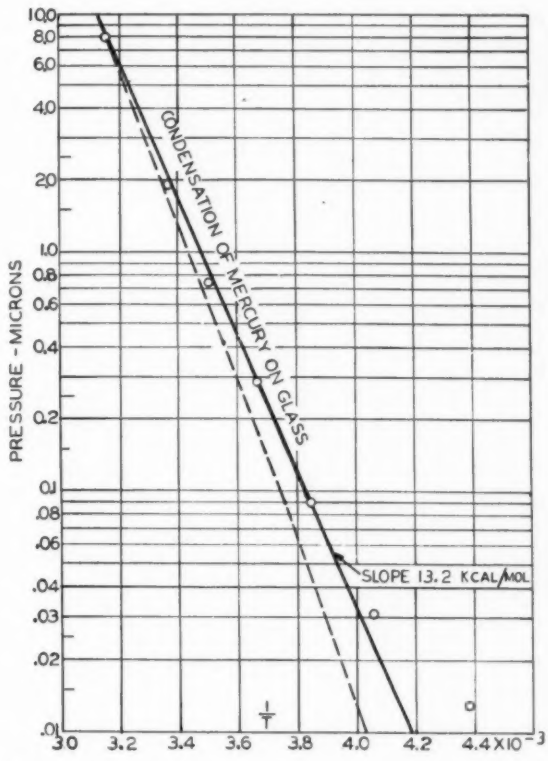


Fig. 2. Pressure of mercury vapor at which condensation starts on glass surface at a temperature T. Dotted line shows saturation vapor pressure of mercury at temperature T.

[4] The adsorption or "clean-up" of mercury ions in a discharge was discovered and studied some time ago by Dr. Carl Kenty, who has described his work in J. App. Phys. 9, 55 (1938).

adsorbed on glass and metal parts. After six hours, the vapor pressure had dropped to 0.25 microns, while at the end of 24 hours, the pressure had further decreased to 0.07 microns. After two weeks, the density corresponded to 0.04 microns, but strong heating of the tube in a flame restored the initial pressure. In another experiment, with the test tube at 97°, the valve was opened for 30 seconds to admit mercury vapor to 1.3 microns pressure. With the gauge on only at momentary intervals so that adsorption of ions would not be considerable, the mercury density decreased gradually for two hours to a steady value only one-half the initial, showing slow adsorption had taken place even at 97°.

Photographic Study of Lightning*

R. E. Holzer, E. J. Workman
University of New Mexico, Albuquerque, New Mexico

AND

L. B. Snoddy
University of Virginia, University, Virginia

(Received November 19, 1937)

A simple method of analyzing lightning photographs taken with moving film cameras is described. The results of analysis of two air discharges with some interesting features and one very unusual type of discharge are presented. Certain discharges at the close of storms and the correlation of electrical and photographic measurements are discussed.

A PHOTOGRAPHIC study of lightning with moving drum cameras in the vicinity of Albuquerque, New Mexico was begun in 1935[1] and continued during the summer of 1936. The present paper is a brief report of some of the unusual results obtained in the summer of 1936.

Apparatus and Methods of Measurement

The photographic equipment consisted of four cameras with stationary lenses, three provided with films mounted on rotating drums, and one with stationary film. In cameras No. 1 and 2 the films moved horizontally with speeds 72 and 780 cm/sec., respectively. In camera No. 3 the film moved vertically with a speed of 500 cm/sec. Camera No. 1, which was slower than either No. 2 or No. 3 was used to indicate the time order of events separated by more than one revolution of the faster cameras, and to indicate the presence of fainter strokes not recorded on the fast cameras. The moving drum cameras, driven by small d.c. motors, were rigidly mounted on the shelf of a portable laboratory which consisted of a steel enclosed room built on a heavy automobile chassis. The lenses were achromatic and of excellent quality; the films were Eastman supersensitive panchromatic. A small generating voltmeter was mounted on the roof of the laboratory to determine the sign and the approximate magnitude of the electrical field intensity. The distance of the flash was determined by timing the interval between the lightning and the thunder.

The time intervals between various events in the lightning flash were determined by superimposing transparent reproductions of photographs from the films of cameras 2 and 3 (or by projecting images of the films on the same screens) so that the respective horizontal and vertical axes of the two reproductions are parallel. Next, a point E_2 from the reproduction of film 2 and a point E_3 from the reproduction of film 3, which represent the same event E in

* Work supported by grant from Penrose Fund of the American Philosophical Society.

[1] Workman, Beams and Snoddy, Physics **7**, 375 (1936).

the original flash are superimposed. Now any other pair of points E_2' and E_3', one from each reproduction, representing any other event E' in

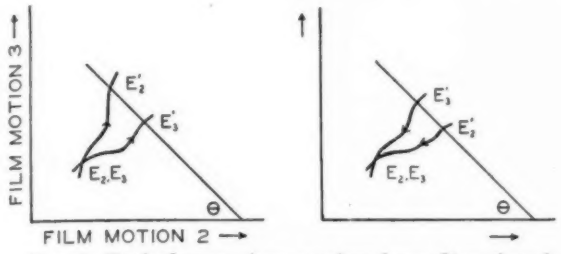


FIG. 1. Both figures show strokes from films 2 and 3 superimposed. In (*a*) event E' occurs after E. In (*b*) event E' occurs before E.

the original flash must lie on a line making an angle θ with the horizontal axis, where θ is determined by the ratio of the vertical film speed to the horizontal film speed. A diagram of the relation of the points on the superimposed reproductions is shown in Figs. 1(*a*) and 1(*b*). The separation of E_2' and E_3' is directly proportional to the time interval between events E and E', and the direction one must proceed from E_2' to E_3' indicates the order of the events E and E'. The method may be used to establish the correspondence of any two points on the two reproductions by superimposing the reproductions as indicated above and drawing a line through any point in one making an angle θ with the horizontal. The corresponding point must be at the intersection of this line and the stroke on the other reproduction.

The drums of the rotating cameras were sufficiently large that a Cartesian coordinate system could be used without introducing sensible errors due to lack of flatness of the film. There is a definite advantage in having two cameras with films moving perpendicular to each other, from the point of view of both photography and analysis. In a single storm characterized by nearly vertical cloud-ground strokes and nearly horizontal air discharges, the repeated elements of both types of strokes are clearly separated. The simple method of analysis is applicable to all discharges, and particularly useful in occasional complicated discharges.

The data on path lengths and velocities of the strokes given below are the projections of the true length and velocity on a plane parallel to the focal plane of the camera. To obtain the probable length and velocity of the strokes in space the values given below may be increased approximately thirty percent as Schonland and others have done.

RESULTS

The summer season of 1936 in the vicinity of Albuquerque was very exceptional in its small number of night storms. However, eight sets of photographs were obtained, five of which were cloud-ground strokes similar in all essential characteristics to flashes reported by other investigators. Of the remaining three, two exhibited some interesting variations of the air discharge, and another was unlike any discharge previously reported.

Flash No. 1 was of interest primarily because of the unusual chain of events it represented. A sketch of the stroke, made from the film of the slow horizontal camera, is shown in Fig. 2. The photographs were taken in the later part of a mountain storm on August 17, 1936. The elevation of the observing station was 5000 feet above sea level. The electric field at a distance of five miles from the active portion of the storm indicated a negative cloud base and a positive upper cloud. The base of the cloud was approximately 0.5 miles above the ground. The flash was initiated by two stepped leaders, similar to those described by Schonland, Malan, and Collens,[2] proceeding along the path AB and CDE with projected velocities of 3×10^7 cm/sec. The leader AB started 100 microseconds before

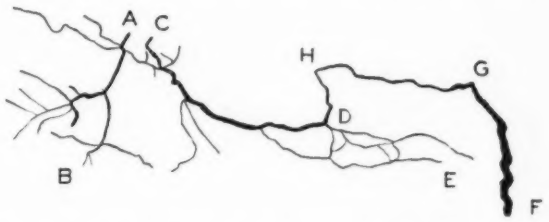


FIG. 2. A tracing from an enlargement of flash 1, film 1.

CDE. Neither discharge reached the ground. 30 and 36 milliseconds, respectively, after the leader AB started, two flashes with velocities between 6×10^8 and 10^9 cm/sec. followed each other over the path AB, both ending in the air.

[2] Schonland, Malan and Collens, Proc. Roy. Soc. **A152**, 595 (1935).

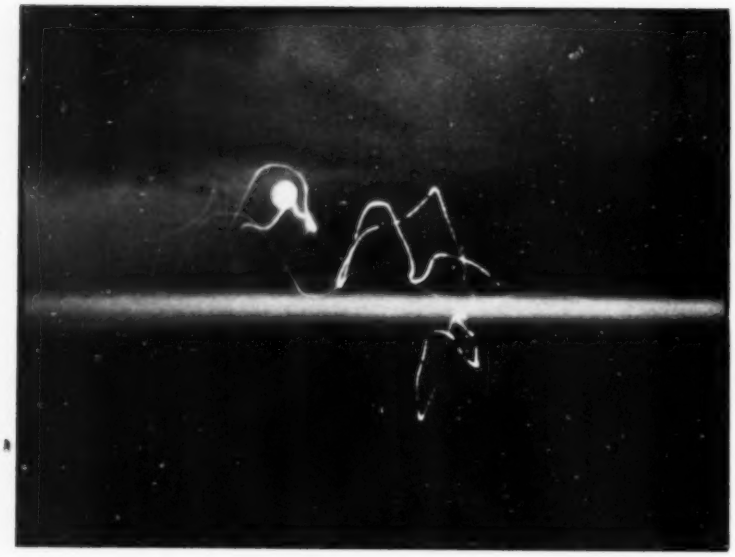


FIG. 3. An enlargement of flash 7, film 2. Film motion is right to left.

83 milliseconds after the initiation of the step leaders, a much stronger stroke started from the ground at *F* and proceeded upward to *G* where it turned sharply and continued to the cloud base at *H*. The velocity from *F* to *G* was 4.0×10^9, and from *G* to *H*, 3.2×10^9 cm/sec. About thirty microseconds later a flash proceeded from the cloud base at *H* to the cloud base at *C* over the path blazed by the step leader *CD* which had been idle for eight hundredths of a second. The absence of a return stroke, immediately following the stepped leader *CDE* is apparently due to the failure of the stroke to reach a large charge reservoir, which is the usual case in air discharges. The ionized path of the stepped leader carried a return stroke only after the potential difference between the cloud base at *C* and *H* was increased by the stroke *FGH*.

Flash No. 6, photographed during a cold front type storm, was a repeated air discharge between one cloud base and the base of another portion of the same cloud over a path of projected length of 0.8 of a mile. The observing station was approximately 7000 feet above sea level, and the cloud base 0.5 mile above the station. The flash consisted of twelve strokes over one path and its branches, the intervals varying from 1 to 33 milliseconds. The total time of the flash was 88 milliseconds. The average projected velocities of the different repeated strokes ranged from 2.7 to 8.0×10^8 cm/sec. The strokes were of the "dart leader" type all moving is essentially the same direction from the first cloud toward the base of the second. No return strokes occurred. The repeated nature of the air discharge is undoubtedly due to the relatively low conductivity and volume distribution of the charge in the cloud as in the case of repeated cloud-ground strokes. Not all of the strokes were of the same length, and in the case of some strokes, only a portion of the path near the middle or the end was illuminated by the discharge. The explanation of this observation is not clear. The stroke may have been continuous from the first cloud to the luminous portion but of intensity so low that it could not be recorded photographically as in the case of some stepped leaders. However, this discharge is not similar to the usual stepped strokes previously reported.

Flash No. 7 is unlike any moving camera, lightning photograph that has come to the writers' attention. Figs. 3 and 4 are photographs of the flash taken on cameras No. 2 and 3, respectively. The photographs were taken on the night of September 3, 1936 at the close of a cold front type thunderstorm in the vicinity of Santa Fe, New Mexico. The observing station was approximately 7000 feet above sea level. Flash 7, together with an intense single flash four miles from the cameras which occurred 100 microseconds before flash 7, were the last discharges of the storm. At the time of a preliminary report[3] on this photograph, it was thought that flash 7 was approximately four miles from the camera, about the same distance as other flashes observed during the storm. Subsequent careful reexamination of the horizon at the location has shown that the flash was much closer than the cloud-ground flashes photographed in the same storm. The flash could not have been farther than 1500 feet, but since no apparent thunder was associated with the flash, it was not possible to determine accurately how far the different parts of the flash were from the cameras. Three

[3] Workman and Holzer, Phys. Rev. **51**, 149A (1937).

subsequent still photographs taken during the summer of 1937 and studied by two of us[4] indicate that the discharge was very likely less than 100 feet from the cameras. Since the moving drum cameras were 18 inches apart stereopticon effects as well as film motion are responsible for the relative distortion of the images on films 2 and 3. Complete and accurate analysis of the discharge is therefore impossible.

Partial analysis of the photographs indicate that the discharge consisted of at least four elements, not obviously connected, which traveled with velocities of the order of magnitude of 10^7 cm/sec. The fact that the persistence of luminosity at any point in the path is not longer than 10 microseconds indicates that the strokes consisted of moving luminous darts, probably similar to the darts in the dart leader stroke. There is a definite possibility that in more than one place the discharge traveled in two directions from a single point. One of the most interesting features of the discharge is the number of very sharp reversals of direction. While the discharge was very close to the ground there is no clear evidence that it "struck" the ground.

The ball-like figure which appears in both Figs. 3 and 4 is probably not what has been called ball lightning. On the original negatives the stroke is clearly defined through this region, and in the printing process some of the contrast has been lost. The ball-like spot is probably ordinary halation due to the fact that the stroke doubled back in this small region, remaining there for a longer time than in other portions of the picture. The horizontal streak in Fig. 3 and the vertical streaks in Fig. 4 are due to the headlights of an automobile on a nearby road which came into the field of the cameras after the stroke, but before the shutters could be closed.

Discussion

Adequate description of flashes 1 and 6 have been given above, but flash 7 and the intense single flash which occurred almost simultaneously with it deserve further discussion.

Flash 7 is apparently a type of air discharge which occurs under very complex electrical field conditions. The sharp path reversals are evidence of intense local fields, probably due to local space charge concentrations.

It is particularly interesting to note that this unusual type of discharge (flash 7) occurred nearly simultaneously with a very intense single flash with cannon like thunder, of which photographs have previously been made,[1] and which characteristically appears, in the vicinity of Albuquerque, at the close of a storm—usually

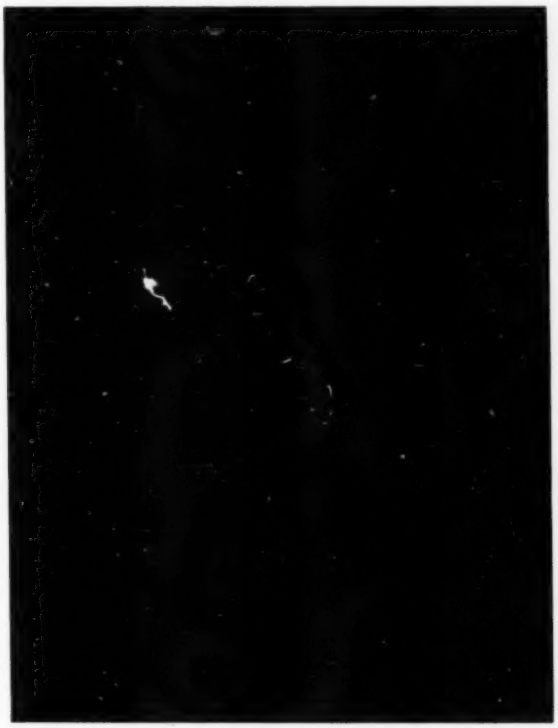


Fig. 4. An enlargement of flash 7, film 3. Film motion is upward.

[4] Workman and Holzer. Two of these still photographs were taken by Mr. W. E. Burk, Jr. within a few hundred yards of the place where flash 7 was photographed. A third photograph was taken by Mr. B. W. Cartwright and published in the August 23, 1937 issue of *Life* magazine. The characteristics of all of these discharges appear to be the same. The paths of all of the discharges were extremely irregular, and close to the ground. In all cases the photographer was apparently less than 100 feet from the discharge. It seems probable that discharges of this sort are not lightning in the usual sense of the word, but are secondary discharges which occur during violent thunderstorms. The irregularity of the path, in some cases complete reversal of direction, suggests extremely irregular electrical fields produced by space charge from corona points near the surface. In at least two photographs the general illumination of objects in the more distant background suggests that these discharges occurred nearly simultaneously with more extensive lightning strokes and may have been started by the rapid charge of electric field intensity which accompanies nearby lightning strokes.

the last discharge—when it occurs at all. While the data are still too meager to justify any generalizations, it has been found that in this vicinity there are a number of thunderstorms with cloud base negative and upper cloud positive at the front of the storm in the region of greatest lightning activity, and with positive cloud base at the close of the storm. The positive charge in the cloud is probably carried toward the ground from the storm front by subsidence winds. It has been observed on more than one occasion that several minutes after most of the lightning activity has ceased, these intense discharges, either single or very close repeaters, occur.

Flash 7 is apparently characteristic[4] of a type of discharge which occurs more frequently than is generally realized. The fact that discharges similar to flash 7 have not previously been obtained on a moving drum camera is probably due to the desire of observers to have an unobstructed view of the storm. As a result, cameras are usually pointed away from nearby objects, on the ground, in the vicinity of which discharges like flash 7 apparently occur. A more systematic attempt to photograph flashes of this sort is planned.

In conclusion we wish to acknowledge with gratitude the financial assistance of the American Philosophical Society and a number of local contributors which has made the work possible. We wish also to acknowledge our indebtedness to Mr. William Burk for obtaining some of the photographs, to Mr. E. L. Hardy, meteorologist, United States Weather Bureau, Albuquerque, for his continued cooperation, and to Mr. Lincoln Koch for assistance in analysis of the data.